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Registered as a newspaper at the
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The olive baboon,
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Refined Carbohydrate Foods and Disease

Some Implications of Dietary Fibre

edited by D. P. Burkitt and H. C. Trowell

June/July 1975, xiv+352 pp., £7.80/$20.75 0.12.144750.2

This important and extensively documented book provides considerable evidence that many western diseases may be related to a reduction in consumption of dietary fibre, which includes celluloses, hemicelluloses and lignin. Hitherto these substances have been almost totally neglected in food technology. With the modern tendency to process foods for convenience, cereal foods in particular suffer from the removal of this fibre which is essential for the fulfilment of certain physiological functions: research shows its deficiency can be one of the causes of such critical diseases as ischaemic heart disease, tumours of the large bowel, diverticular disease, gallstones, appendicitis, varicose veins, haemorrhoids, obesity and diabetes.

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edited by C. A. Stace

June/July 1975, £14.80/$39.25 0.12.661650.7

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The Biology of the Male Gamete

Supplement No. 1 to the Biological Journal of the Linnean Society, Volume 7, 1975

edited by J. G. Duckett and P. A. Racey

June/July 1975, xviii+590 pp., £15.00/$39.75 0.12.223050.7

This symposium aims to bring together diverse aspects of the biology of the male gamete of animals and plants, to relate their structure to their function and to establish the extent of common ground between them. The book, which is extensively illustrated with many previously unpublished micrographs, contains up-to-date reviews coupled with the results of recent research by recognized specialists. The treatment of the plant kingdom is especially comprehensive with papers on the male gametes of algae, fungi, archegoniates and angiosperms. Contributions on animal gametes deal not only with topics of perennial interest such as motility and survival, but also with recent research on sperm proteins and enzymes.

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Volume 2

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Crystal Growth from High-temperature Solutions

D. Elwell and H. J. Scheel

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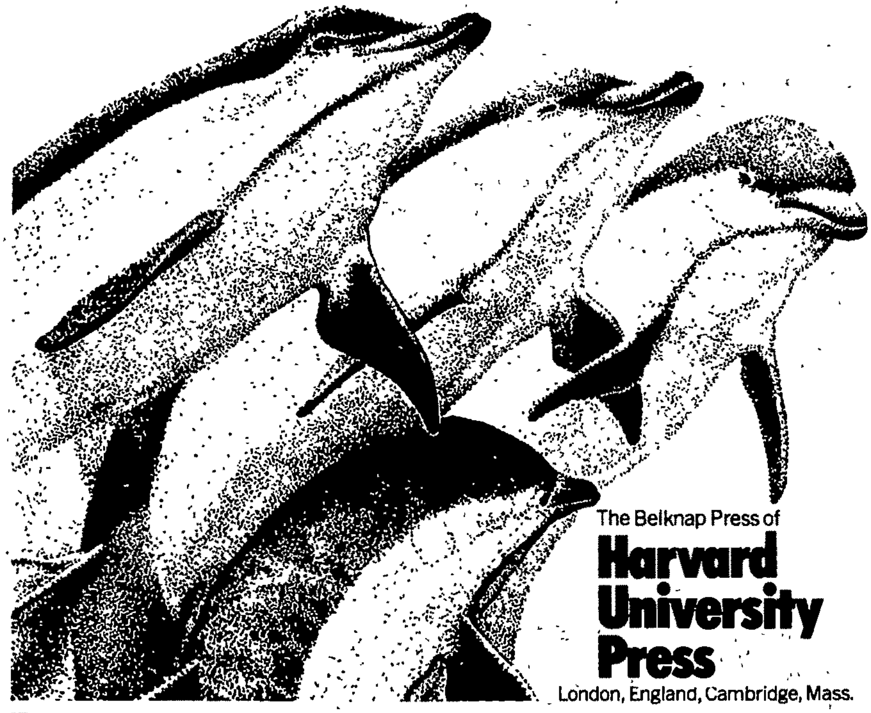

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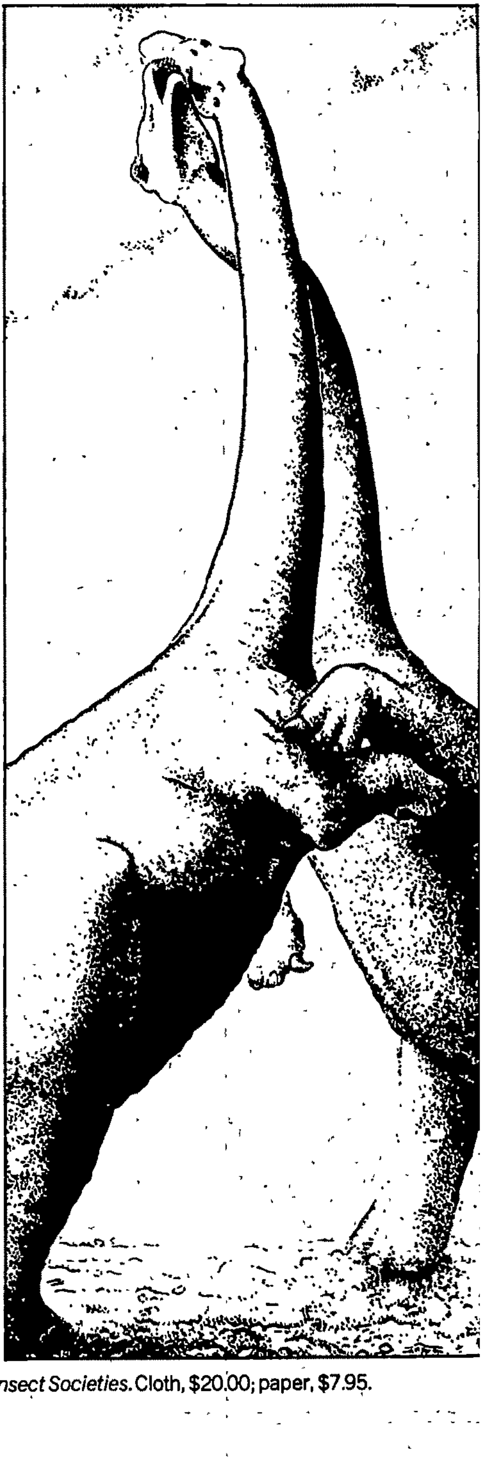

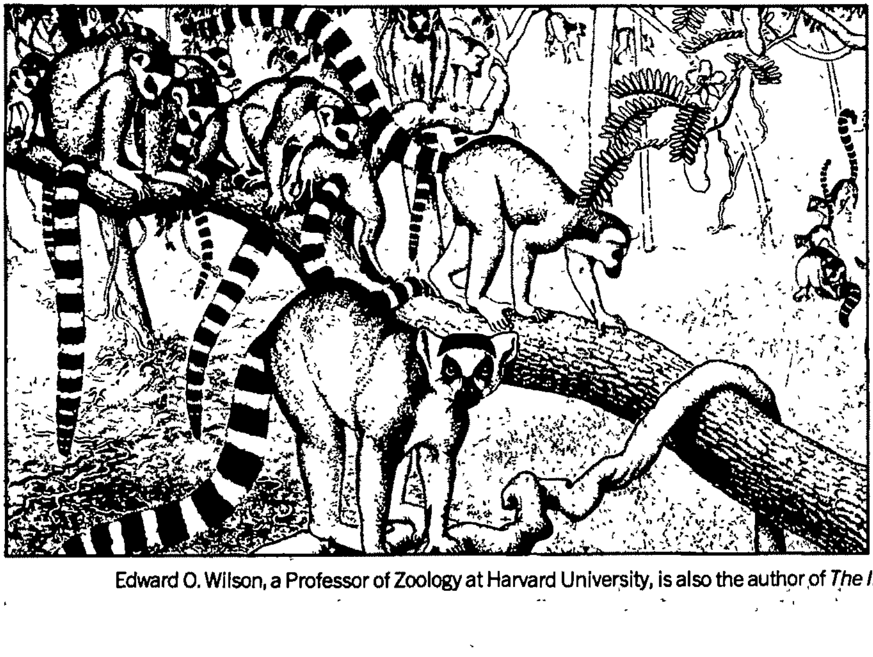

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Vol. 255 No. 5506 **May 22, 1975**

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Published weekly
ISSN 0028-0836
Registered as a newspaper at the
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Nature looks at
Scientific manpower (pp 284–305)

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ISSN 0028-0836
Registered as a newspaper at the
British Post Office

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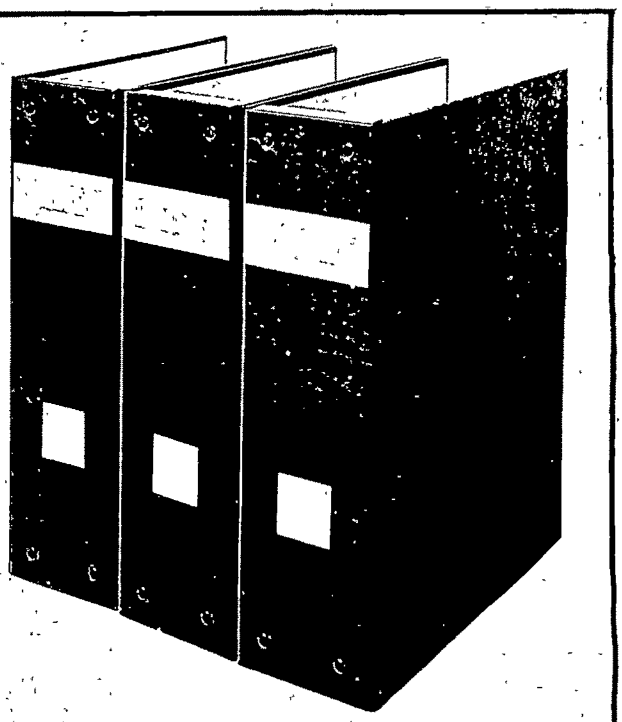

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G. E. Sladen: Methods of studying intestinal absorption in man. G. E. Sladen: Absorption of fluid and electrolyte in health and disease. H. B. McMichael: Absorption of carbohydrates. M. D. Hellier and C. D. Holdsworth: Digestion and absorption of proteins. M. L. Clark and J. T. Harries: Absorption of lipids. C. D. Holdsworth: Calcium absorption in man. B. Brozovic: Absorption of iron. I. M. Coupar and Ian McColl: The pharmacology of absorption. Index.

Man-Made Lakes and Human Health

edited by N. F. Stanley and M. P. Alpers

June/July 1975, xvi+496 pp. £12.40/$32.75 12.663550.1

The contributors, concerned by the effect of man-made lakes on human health and the general ignorance of this relationship, present the ways in which dams produce gross and sudden interference with established ecosystems. The articles are divided into four main sections—health problems, specific geographic areas, ecological factors and the human aspect. Emphasis is placed throughout on the mistakes of the past, the dangers of the future and the importance of international planning and co-operation. By defining the basic problems involved, illustrating the need for further action, indicating possible solutions, providing lines for further research, and outlining the nature of future effective action, the contributors hope to provide sufficient accurate data to enable Governments, Public Health Authorities and the scientific community to make intelligent decisions about man-made lakes.

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Vol. 255 No. 5508 **June 5, 1975**

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ISSN 0028-0836
Registered as a newspaper at the
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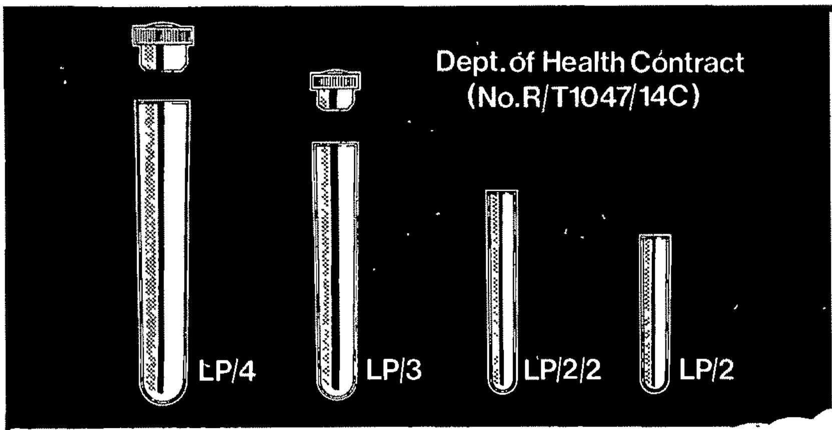

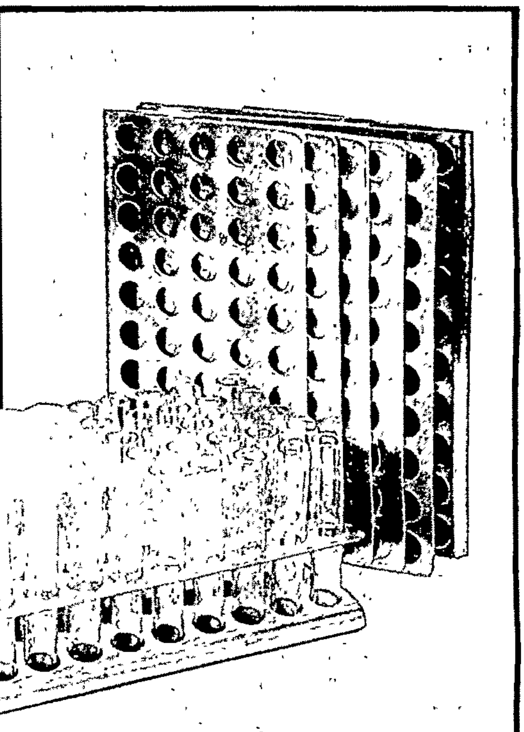

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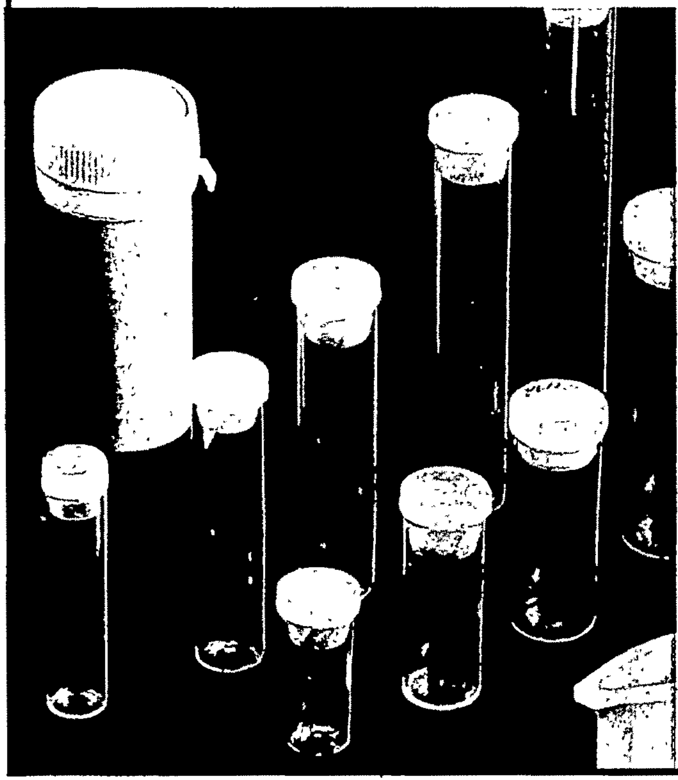

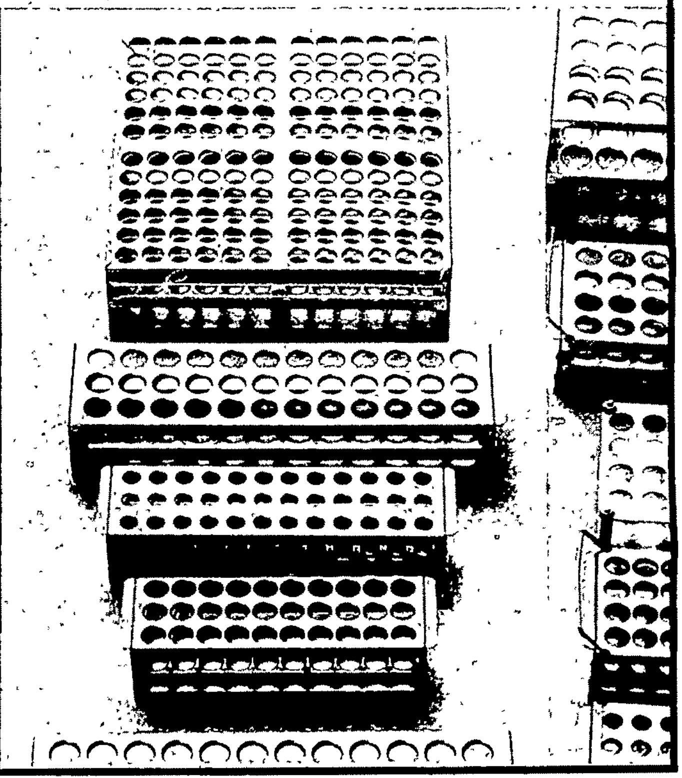

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Published weekly
ISSN 0028-0836
Registered as a newspaper at the
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nature May 1, 1975

Science policy must keep pace with science

For the past few years the Organisation for Economic Cooperation and Development (OECD) has been brooding on the so-called research system—the network of organisations which support and do scientific research, be it fundamental or applied, military or ecological. Eight European countries, Canada and the United States have been subjected to an examination of how they run their science, and the last report rounding off the series with conclusions and a string of recommendations is recently out. At least, the book version is newly published—it has been circulating in *samizdat* in varying degrees of confidentiality for the best part of a year (*The Research System,* **3**; OECD Paris, £3.80).

The problem with reports from international organisations is that they are bound to be toothless, and therefore less than compulsory reading. Nothing fixes the mind more when reading a White Paper than the knowledge that the author is committed to converting proposals into reality within a few months. An OECD document can be dismissed as reflecting the opinions of a staff with necessarily limited access to information and no powers to implement proposals. To do so with this document, however, would be to fail to appreciate the value of a relaxed international perspective on the remarkable phenomenon of the turning of various scientific tides around 1970 and the attempts (and sometimes failures) of scientists and administrators to cope with the new scenery of the 1970s. The report, and particularly the conclusions, dry as they may seem, could with profit be read by any scientist who worries about what he will be doing in five years time.

Part of the OECD's theme is that the pursuit of major strategic objectives, particularly in defence, nuclear energy and space, started to falter in the late 1960s and since then the main running has been taken up by matters of more common concern both nationally and internationally, such as the environment, community services and health. Few would doubt this, though for good measure graphs are included which show among other things that in the past ten years public expenditure on health research and development at least doubled in real terms whereas defence expenditure remained static. Indeed in Canada research and development on health has risen eight-fold in ten years, and is now as great as research and development expenditure on defence.

With the decline of the "major strategic objective" as a dominant force in science, however, have the mechanisms for evolving science policy changed accordingly? For the OECD sees several sacred concepts of the 1960s as being no longer accepted as axiomatic.

- The Humboldt principle that teaching and research are inseparable "may have done more harm than good" by prejudicing the evolution of research and its vitality as much as prejudicing teaching.
- The search for a 'Magic Figure' for the percentage of GNP spent on research and development has lost much of its meaning with the manifest scientific and technological successes of small-spending countries.
- The idea that fundamental science should have a certain percentage (often quoted as 10%) of total expenditure on research and development devoted to it is 'little more than folklore'.
- The concept of peer selection for the disposition of money for research to a principal investigator "does not seem to have guaranteed the quality of research so much as it has consolidated its regular advance along beaten tracks".

By this analysis the scientific community found itself caught short by the change in priorities imposed on it and tended to rationalise the swing away from the hard sciences among students as "an easy and even amateurish choice inevitable in a mass university open to all". But maybe these young people had a premonition of the future requirements of society; if sustained long enough the premonition would become self-fulfilling.

The changes that science and technology have undergone and will continue to undergo are not such as will throw thousands out of work, and they are not immediately detectable by scanning the scientific literature for a discontinuity of some sort. Nevertheless they are profound in that the earliest effects appear amongst science's latest recruits—and science has traditionally depended on the young. They are also profound in that they move science into a more political environment and a world in which there are nothing but complex answers to the simplest of questions. Can science-policymaking cope with the transition and can the sort of institutions established under past policymaking (or lack of it) fulfil a continued useful role? Two of the OECD's points deserve more debate in the British context.

First, the arena in which the support for and use of science is discussed should be broadened to permit the participation of "the young, the quiet, the not-so-eminent". Academics, foundations and learned societies should be encouraged to "equip themselves with the means for participation". Second, the countries which OECD believes have tried best to adapt to new dimenisions have been those where ministries have been constituted with wide responsibilities for "coordinating and animating scientific effort"—Science Ministries, without other sectoral responsibilities.

The need for a science ministry is not immediately apparent in Britain, especially at a time when many scientists are still readjusting to the organisational changes of the last three years. But the need for somewhere to discuss how the management of science should be conducted in 1980 is all too obvious; not in a one-day meeting but as a regular open forum where the young, quiet and not-so-eminent can learn and be heard. Council for Science and Society, please note.

Malaise of clinical organ grafting

Professor Roy Calne, of Addenbrooke's Hospital, Cambridge, considers why organ grafting in the UK is in the doldrums.

MORE than 10 years ago an immunosuppressive regimen of the thiopurine Imuran, together with corticosteroids, was introduced and clinical organ grafting became possible. The actions of these agents are not fully understood; they can be toxic and sometimes they are totally ineffective at the maximum tolerated dosage. Nevertheless more than 20,000 kidneys have been transplanted in patients treated with Imuran and steroids, and the results are far from discouraging, especially in allografts between close relatives. Rejection remains the chief barrier to progress, but the quality of the organ when grafted is also very important. In grafts between identical twins where there can be no rejection and the organ, coming from a live donor, should be in perfect condition, there is an 86% chance of the graft sustaining the patient's life at two years and the longest period of survival after operation is now more than 18 years. If a kidney is grafted from a blood relative who is not an identical twin, the figures fall to 70% and 15 years, and with a cadaver donor (in which case both rejection and the condition of the organ are likely to raise problems) the figure is 48% and 11 years.

Two inter-related factors are responsible for the present malaise, namely the lack of a significant advance in the control of rejection and the poor therapy offered to patients, compared with what could be possible using existing techniques.

Then years ago the mood of surgeons and immunologists was full of optimism. They felt that if results such as those described above could be obtained with the earliest immunosuppressive agents investigated, surely more effective and less toxic drugs would soon be available. Moreover, immunology, which had been stagnating during the previous three or four decades, was given an immense boost. The work of Medawar and his colleagues led to the successful recruitment of brains and funds into transplantation biology. As had been anticipated, this focus of interest soon bore fruit in the form of knowledge of cellular and humoral immune mechanisms. How disappointing it was to realise that this new light revealed that phenomena that had seemed simple were in reality excessively complicated and that almost every attempt to apply successful experimental techniques of graft prolongation in inbred rodents to outbred animals including man led to failure. As Medawar has stated the "tyranny of the skin graft in the inbred mouse" held back progress in organ grafting. Allografted skin does not behave like vascularised organs, and the controlled parameters of an inbred rodent strain do not resemble the immense variation of antigenicity and immunological response seen in man.

Much hope seemed justified when it was shown that antilymphocyte serum prepared in the rabbit against mouse lymphocytes would prevent the rejection of skin grafts from widely disparate donor strains and even from other species. Yet, many years after its first application to patients with kidney grafts, it is still not clear if antilymphocyte globulin (ALG) therapy adds significantly to the therapeutic index of Imuran and steroids; there is no doubt, however, that some batches of ALG can be dangerously toxic and can potentiate the risks of infection especially to viruses and fungi.

Tissue typing was another endeavour which seemed to have a bright future. A new and very complicated system of serologically defined antigens present on lymphocytes and many other tissues was discovered. They are certainly relevant to the outcome of graft survival. The genetics of this system and even the chromosome responsible have been demonstrated elegantly. Nevertheless complete matching of these tissue types in siblings only permits survival of skin grafts for some 20 days compared with 7 to 10 days when the grafts are mismatched. It is clear that there must be other important antigens that are not being detected, since the grafts are always rejected. Application of tissue typing to clinical kidney grafting has also been disappointing, except in grafts between siblings. If donor and recipient siblings are of identical tissue type, there is an 80% chance that the transplant will function normally after two years. Fully matched grafts between unrelated people do much better than unmatched grafts, but the influence of typing between unrelated donor and recipient when the match is only partial is weak, and most surgeons pay more attention to the clinical considerations and the quality of the organ than to the matching. Tissue typing and direct cross matching of recipient serum against donor cells is of importance in patients previously sensitised to transplantation antigens as a result of rejecting a previous graft, receiving blood transfusions or following pregnancy. Sensitised patients must receive a kidney from a donor to which he does not already possess heightened immunity, otherwise aggressive rejection is likely. As more transplantation antigens are defined by new techniques, tissue typing will become more relevant, but in practice the likelihood of finding a perfectly matched donor organ will become increasingly difficult.

Most workers have felt that the ideal form of immunosuppression would be specific only for the donor in question, so that the patient would not be subjected to increased risks of infection. Laboratory models of specific 'desensitisation' using transplantation antigen and antibody preparations have been described, but to date there has been little success in their clinical application. It may be necessary to monitor the reactivity of the recipient towards donor antigens in order to treat the patient with an effective 'recipe'. Such speculations are for the future, but it is important to remember that half of the patients receiving random, unmatched, unrelated cadaver kidneys do well and since the dosage of immunosuppressive agents can often be reduced to relatively safe maintenance levels, it is likely that these patients have come to terms with the graft antigens to allow for a peaceful and profitable co-existence that probably involves natural donor-specific unreactivity.

In spite of this static background, clinical organ grafting is a worthwhile form of medical treatment that is not yet available to many patients in need. To be dying from failure of a vital organ function is as serious an affliction as any other lethal disease. When we spend huge sums of money without question on the treatment of cancer and operating on the elderly, why should organ grafting be attacked so often as an expensive luxury? Since organ grafts are seldom successful in those over the age of 50, most patients in need are of potential economic value to the state. Dying slowly in an expensive hospital bed is likely to cost far more than a transplantation operation which, if successful, will restore a breadwinner or housewife to the community. These financial matters do not take into consideration the suffering of the patients and their relatives if they are denied treatment. In the United Kingdom there are approximately 3,000 deaths a year from kidney disease in people aged five to 55 years. The DHSS has established 25 dialysis and transplantation centres. Most of the dialysis spaces are full and new patients cannot receive treatment since only 500 kidney grafts a year are carried out. The majority of transplantation centres are performing less than half the number of grafts for which there are facilities. This is demoralising for the staff and patients, especially since the cause—namely a desperate shortage of donor kidneys—is potentially so easily soluble.

A kidney can be grafted from a live volunteer or a cadaver. Since the results of grafts from cadavers differ little from those obtained with grafts from unrelated live volunteers, only blood relatives are considered as donors. The operation for a live donor is not trivial and very often there is no suitable or willing familial donor. Removal of an organ from a dead body can do the deceased no harm and can be a gift of life to the recipient. Why then is there such a serious shortage? It has been suggested that in England the only thing more sacred than a live dog is a dead human body. This feeling for a cadaver may be an important subconscious reason for the lack of enthusiasm for organ donation after death. The precise details of what befalls a corpse are conveniently thrust out of conscious deliberation. Thus there has never been any serious opposition to the autopsy examinations ordered by coroners following most accidental deaths. The wishes of the deceased in his lifetime and those of his relatives have no bearing on the decision. All the organs are removed and examined, but these matters are not discussed by the relatives or the public. A request for organ donation does, however, require conscious thought on an unpleasant topic—death.

There are two categories of cadaver donor:

- Bodies brought into hospital dead. Here the diagnosis of death is straightforward, but there is urgency in removing the kidneys since if they are left in the corpse for more than an hour they will be useless. Once removed they are cooled and at 0 °C they can be kept in good condition for 12 hours. If they are perfused continuously with an appropriate cold oxygenated solution they can be preserved for up to 48 hours.

To comply with the Human Tissue Act of 1961, if the wishes of the deceased are known they are followed, otherwise the relative's permission must be sought. Often the relatives cannot be found in the limited time available. If they are traced it may be cruel to raise the question of organ donation at this moment of terrible distress; although sometimes this is not the case and the feeling that some good may result from their tragedy can give comfort. It is obviously preferable to know the wishes of the deceased—the simplest practical solution is to carry a kidney donor card as supplied by the Department of Health and Social Security. In spite of the fact that most people are in favour of organ donation after death, I doubt that more than a small percentage would carry a card, since it is a bother and its presence is a continuous *memento mori*.

- A body with a dead brain but an intact circulation, maintained by mechanical ventilator of the lungs. This category of cadaver donor is the only type from which heart or liver grafts can be used, because of their extreme vulnerability to ischaemic damage. After death of the brain, other organs and tissues die at different times depending on their oxygen requirements, unless the circulation is artificially maintained by oxygenating the lungs. Obviously all vital organ grafts must be alive, otherwise they would be useless to the recipients. The organs of a decapitated body could be perfused and oxygenated with the heart beating, yet no one would consider the individual to be alive. Nevertheless there is so much superstition and folklore concerning the heart that many people consider the beating heart to be the cardinal sign of life.

How can this view be reconciled with the fact that it is routine surgical practice in open heart operations to stop the heart so that it can be repaired? During this period the patient is kept alive because his brain is perfused by an artificial heart–lung machine. Should the cerebral circulation fail, the patient will certainly die. Patients with brain damage from an injury, haemorrhage or tumour are

Kidney Donor Card

Your kidneys could help someone to live after your death

Keep the card with you at all times in a place where it will be found quickly

THE Department of Health and Social Security has recently stepped up distribution of kidney donor cards like these. Four million have been distributed, and although this does not mean that a similar number have been completed, hospital authorities are finding that an increasing number of emergency cases, suitable for kidney transplant or research, are carrying the cards. Area health authorities are responsible for distribution, and the cards can usually be obtained from local hospitals.

I (full name)
request that my kidneys be used after my death for transplantation or, if they are found unsuitable, for research.

Signature of donor Date

The above-named person has discussed his/her wish with me and I do not object to that wish being complied with.

(Name of next-of-kin)
of (address) ____________________
____
Tel ____________
Signature of next-of-kin ____________ Date ________

resuscitated and assessed. If the damage is severe they stop breathing and this function is taken over by a mechanical ventilator. If after careful examination by experienced neurologists or neurosurgeons it is established that the brain is totally and irreversibly destroyed, then the ventilator is stopped since the perfusion of a corpse can be of no benefit to the deceased and would cause distress to his relatives and the nursing staff.

The decision to stop the ventilator has absolutely nothing to do with organ grafting. This was an accepted humane procedure before organ grafting was established. Once the decision has been made, however, and if it is likely that the case may be a suitable organ donor, there is then time to discuss the matter unhurriedly and sympathetically with the relatives, and the transplantation team can be prepared. In the USA and several countries in Europe, the organs are removed before the ventilator is stopped, since the intact circulation will ensure that the organ is in perfect condition. In the UK organs are usually not removed until after the ventilator has been stopped and the circulation has ceased. The heart may beat for up to an hour without oxygen; during this period all the organs including the heart are suffering severe damage — since the blood passing through them is becoming progressively depleted in oxygen and nutrients and is becoming increasingly acidic.

The British Tranplantation Society recently published a report on the shortage of organs for transplantation, (*Brit. med. J.*, **1**, 251; 1975). It was concluded that apathy in the medical profession was the main cause of the shortage, but that this was aggravated by lack of public information, ambiguities in the law and an unhelpful attitude of some coroners. The report proposed that there should be minor changes in the law, and a code of practice to be followed by all transplantation centres.

Although the main clinical application of organ grafting has been the kidney, both the heart and the liver have been transplanted successfully and have restored moribund patients to normal lives. There are no substitutes for heart and liver function comparable with the artificial kidney. This lack of artificial organ support has held back progress. In spite of this handicap, Dr Shumway in Stanford has transplanted more than 70 hearts with results which are little inferior to those of kidney grafting. There are far more potentially suitable cadaver organ donors than are needed, but sufficient organs are unlikely to become available until it is assumed that after death it should be a routine procedure to remove organs for grafting. ☐

KENNETH MELLANBY

Paper chains

I FOUND the recent white paper *Food from Our Own Resources,* issued by the Minister of Agriculture, disappointing (Cmnd 6020, HMSO) It makes no provision for the possible effects of a fall in the value of the pound sterling, which would make it impossible for us to continue to import food at the present rate, nor does it suggest the action that would be needed if a world food shortage threatened our imports. It does, however, make modest proposals for increasing our agricultural productivity—by enlarging our dairy herd, increasing the acreage under cereals, and growing some oil seed. Even if all targets are met, we will still be far from self-sufficiency and (at present prices) our balance of payments deficit will still be enormous.

The White Paper does imply that a greater improvement in yield and in our finances may result from research. Those scientists remaining in agricultural research (and whose work, it is hoped, will have this result) may, however, be surprised to read that the "National reorganisation [of research] in line with Lord Rothschild's recommendations is already taking effect in the closer integration of research and development with agricultural progress." Although the precise meaning of this curious example of Whitehall prose may be difficult to disentangle, it presumably means that agricultural research is becoming more and more effective. The sad thing is that the writer of the White Paper probably believes that this is true.

It is perhaps too soon to analyse the total effect on research—and particularly on agricultural research—of the Rothschild system, but it is disconcerting to find that there is such a divergence of views between the actual researchers themselves, and the administrators and committee members concerned with scientific policy. In our laboratories we see the number of the scientists at the bench decrease, and find the vacancies of those who leave (often to move into administrative posts) remaining unfilled. Morale is lower than ever before, and complaints of time-wasting paper work, often at the behest of unnecessary committees, are widespread. Lord Rothschild criticised the research councils (to my mind, rightly) for wasting too much time and resources on committees, yet he has, no doubt unwittingly, caused the time spent on committees and working parties to be at least trebled.

The central feature of Lord Rothschild's proposals was the "contract". Money previously paid by the government to research councils has been transferred to executive departments who then pay the council for doing some particular piece of research. The idea was that this would make sure that work was concentrated where it was most needed. Now had contracts been the simple things suggested by Lord Rothschild, involving large sums to be spent on some wide field of work, the conditions simply and briefly set out on 'half a sheet of paper', the idea might have had some merit. But, unfortunately, the Civil Service has seen to it that many contracts are small, complicated and requiring the scientists involved to produce detailed 'project plans' and a constant flow of information to feed the computers which are playing an increasing part in sterilising originality at the laboratory bench.

The saddest result of all this administrative complication is that it has destroyed the enthusiasm of many research workers and has encouraged others to transfer to the administrative posts which seem to be so much more esteemed by the authorities. In the past, research for many was a way of life and workers often successfully chose problems which they believed were relevant and soluble. They were sometimes right, and they were then able to do much to see that their results were used, for instance, by the farmers. The trouble about the contract is that the scientist who has to carry it out may think it is for work which he cannot fully believe in. And he may well be right. But although in theory there are ways in which contractors can be persuaded to use their money wisely, few research workers believe that they will be very popular if they constantly shew that the authorities are incapable of selecting the best problems for investigation.

It is perhaps surprising that, though we have made many changes in the organisation of government-financed research in Britain, no one seems to have tried to find out how these have affected its efficiency. The impression certainly exists that no one is looking at all critically at the results of Lord Rothschild's recommendations. Surely someone should place a contract for such a study. ☐

international news

Out of sight out of mind?

from Vera Rich

PLANS are in hand for a permanent international organisation to be set up to investigate reports of "psychiatric inquisition" (the abuse of psychiatry for political ends) and to take appropriate measures to condemn and prevent such abuses. This is the chief outcome of a symposium on "Medical ethics and abuses of psychiatry for political ends", held in Geneva on April 19, under the auspices of Amnesty International.

After the presentation of case histories of such abuses, and extensive discussions of the moral, juridical and social issues involved, the symposium resolved to set up an initiative committee leading to the establishment of the proposed international organisation. It also appealed to "all medical associations and conferences not to hesitate, in spite of the threats of withdrawal by member countries, [to condemn] explicitly these abuses of psychiatry and to boycott doctors and organisations who lend themselves to them and who refuse commissions of enquiry".

The problem of psychiatric abuse was carefully distinguished from the "errors and cases of negligence" which occasionally occur in any country or system. The symposium was concerned with interventions by a political system to use (or abuse) psychiatry to serve its own ends, by detaining in mental institutions critics of the regime, whose views are considered "at one and the same time, to be both symptom and proof of mental illness", and the use of allegedly curative procedures as a means of political repression.

Although it was stressed in the opening speech that, in accordance with the aims of Amnesty International, the symposium was of an a political and humanitarian nature, the case histories and evidence presented all related to the Soviet Union, since it is from there that most of the evidence comes, both as regards the number of persons concerned and the sophistication of the techniques used. Two former detainees of this kind, Viktor Fainberg and Viktor Nekrasov, spoke of their own experiences in Soviet penal psychiatric institutions. Another Speaker, Dr Marina Voikhanskaya, who was allowed to emigrate from the Soviet Union only a fortnight before, spoke of her own experience as a psychiatrist in a Leningrad hospital, where she had refused to permit the punitive administration of drugs to dissidents temporarily confined there, en route to or from a penal psychiatric institution.

Voikhanskaya and Fainberg: released

The picture she gave was essentially one of a conspiracy of silence. She herself had been practising as a psychiatrist for almost ten years before she came by chance into contact with one such dissident detainee—the artist Ivanov. When she attempted to make further enquiries about such cases she found on the one hand obstruction from hospital authorities and on the other a pusillanimous attitude from fellow psychiatrists, "who did not want to know about such matters". She managed, over the course of four years, to build up a fairly comprehensive picture of the certification of dissidents, under such diagnoses as "creeping schizophrenia", or "reformist tendencies". 'Treatment' in such cases included close confinement, tying the "patient" to his bed for several days "with the neglect of the elementary rules of sanitation", and the administration of massive doses of drugs, notably haloperidol, which would not be justified even had the original diagnosis been correct. Attempts to break through this barrier of silence by Dr Semeon Gluzman and Vladimir Bukovskii, who tried to inform public opinion abroad and (by way of the *samizdat* network) within the Soviet Union, earned them heavy prison sentences. Dr Voikhanskaya stressed that such abuses affect not only the status of psychiatry within the Soviet Union, but the "honour of the entire psychiatric profession".

Yet so long as the penal psychiatric institution remains a useful oubliette for dissidents, there exists the tragic possibility that what was originally a paper diagnosis for political reasons may become, under 'therapy', the simple truth. This, it would seem, is the fate of the Ukrainian mathematician Leonid Plyushch. Since his arrest in 1972 he has spent a year in prison and more than two years in the "Dnepropetrovsk special" (penal) psychiatric institution. According to his wife, Tatiana, Plyushch entered the hospital a sane man and is under the course of intensive treatment, with ever increasing doses of drugs. He suffers petty but cumulatively distressing harassments, and is rapidly deteriorating intellectually and emotionally. "The mathematician Plyushch", she writes, "the Leonid Plyushch known to me, to his children, relatives and friends, this Leonid Plyushch no longer exists. There is an exhausted man, driven to the last brink of suffering, losing his memory, and his ability to read, write, think . . . "

Dr Voikhanskaya characterised the purpose of the 'psychiatric inquisition' as two-fold: the long-term removal from circulation of those who steadfastly maintain views which oppose the established order, and the discrediting of those views among those who, not knowing the dissident personally, may well believe that a diagnosis pronounced by some eminent doctor cannot be entirely unfounded. The case of Leonid Plyushch, however, indicates a further outcome; the dissident, once confined and subjected to treatment prescribed for political not medical reasons, may well deteriorate into a condition of genuine psychological illness—that once out of sight in a suitable institution, he may well become, not only legally but also in fact, 'out of mind'. ☐

Satellites over the oceans

from Angela Croome

THERE have been several moves in recent years to have marine communications carried out by satellites—either of the active type, for navigational purposes, or as passive relays to improve safety services and routing operations. The most developed initiative has come from IMCO, the International Maritime Consultative Organisation, an agency of the United Nations, which is at present holding a 2½-week international conference at its London headquarters aimed at setting up an international maritime satellite executive organisation — INMARSAT — to bring about such a system by the end of the decade.

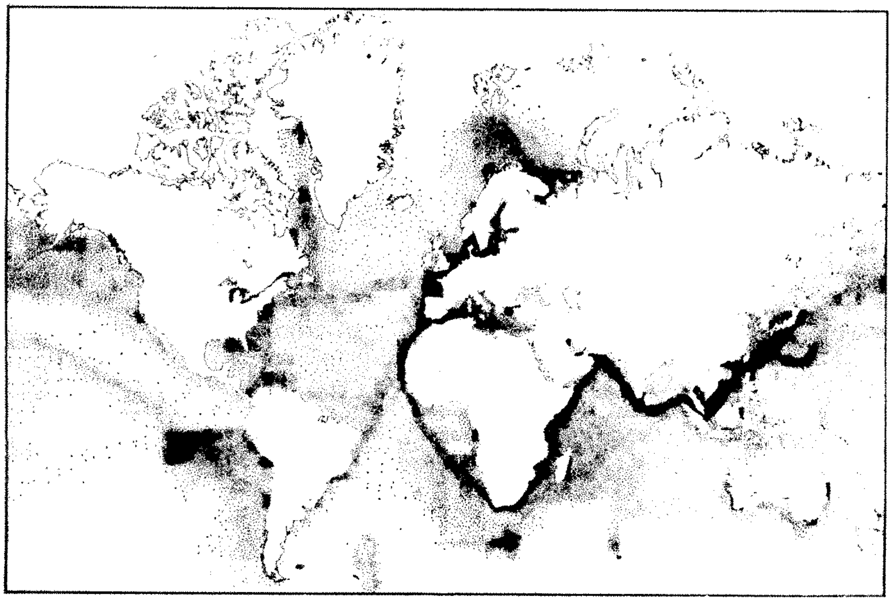
There are a number of reasons for favouring a transfer of ship-shore and ship-ship communications to satellites. Traffic statistics of coast stations reveal that demands for long range h.f. communications, and particularly radiotelephones, are steadily increasing; saturation of existing facilities is expected by 1980. (At some times of day this is already the case.) The size and number of the world's commercial fleet is also going up exponentially—6,000 vessels over 10,000 tons in 1970 against an estimated 60,000 in 1980—and this will add to the overload. The present average delay in international h.f. telegraph traffic is six hours with delays of 12 hours being not uncommon—hardly satisfactory when an emergency occurs. The present scarcity of ships' radio officers today (typically there is only one aboard) means an 8-hour watch which may not coincide with the times of best radio propagation. The vagaries of ionospheric propagation introduce radio blackouts, interference and fading which further limit effective radio time and traffic.

The International Marine Consultative Organisation began considering the possibilities of a switch to satellite communications for ships at sea in 1966. In 1971 frequencies were set aside for the use of maritime satellites by the international radio authorities, and in 1972 a Panel of Experts was set up by IMCO to look at the characteristics and operational requirements of a "maritime mobile satellite system". This met six times and the experts' report—136 pages of it—is the principal document for discussion at the present conference. Its main conclusion is that there is "an urgent need" for satellite communications for shipping and that such a system should concentrate on general communications requirements to handle distress, safety and public correspondence. Radio determination of a ship's position is feasible and attractive but of less priority. The report stresses that a marine satellite system would only be effective and economic if it could carry out more than one function, preferably "as many as possible".

The report considers in some detail the technical requirements of the system, whether it could be combined with the projected aeronautical mobile programme (Aerosat), the economics and a timetable. It comes out in favour of a three-synchronous-satellite system (which could cover all but 20% of shipping) and costs are based on the use of a Thor-Delta launcher. If the report is adopted by the conference there is a Draft Convention appended to bring the new INMARSAT organisation into being. Consideration was given to using the Intelsat organisation (the authority for the existing world satellite communications network) but the poor representation of some of the major shipping countries in Intelsat militates against this. All countries must accept the INMARSAT system it is stressed, because safety at sea cannot be partial.

The US delegation introduced a note of disharmony in advance of the conference, and has been the sole IMCO member to reserve its position on the report. The USA has put on the record that "the establishment of a new international organisation is likely to pose problems . . . leading to serious delays"; it finds inadequacies in the proposed draft agreement and in the economic review (which does not include a cost-benefit analysis) and has doubts that the reliability of ship terminal equipment has been fully investigated. ☐

Map showing the distribution of merchant shipping expected by 1980.



Getty hands out cash

MR JOHN PAUL GETTY, the octogenarian oil millionaire, has set up a $50,000 wildlife conservation prize and is so pleased with the first award that he is now offering it for a second year. The first John Paul Getty Award for Wildlife Conservation was recently presented (at the White House) to the colourful Peruvian, Felipe Benavides, better known as "Mr Vicuna". He has been largely responsible for saving the remaining vicunas of the Andes from extinction and has now established a reserve for them in their native habitat where there are hopes that they may become semi-domesticated. Benavides has transferred the whole of his prize money to running the Paracas Reserve. When not saving the vicuna (and he is now pressing for a ban on whaling within the 200-mile zone of Peruvian waters) Benavides is a business man, and a rather special one since he has the capacity to remain influential whatever the current complexion of Peruvian politics.

Mr Getty is anxious to gain the widest number of candidates for the second award of his $50,000 prize. More than 500 people applied for the first. The fact that Commandant Cousteau was invited to speak at the announcement of this year's award rather suggests that there is an interest in finding a candidate active in marine conservation. The jury for the award (which is chaired by HRH Prince Bernhard of the Netherlands) has been cunningly chosen from the best known figures in conservation including Sir Peter Scott and Cousteau himself, which forces a search for new faces and names in this field. Nevertheless there is a certain irony over the application of this money to foster marine conservation in view of the damage done to sea birds and other marine life by oil spills; it is one that Mr Getty half-acknowledged at his reception. ☐

SIR Frederick Dainton, Chairman of the University Grants Committee (UGC), was among friends last week when giving evidence to the Science Sub-committee of the Commons Select Committee on Science and Technology. No one disputed that he had real problems and no one seemed disposed to pick a quarrel with him over the way he was trying to keep university finances going. Sir Fred warned in his written evidence that the equilibrium of the dual system was in some difficulty because the erosion of UGC support for the base had been more rapid in the past year or so than erosion of funding for the research councils.

The UGC's problem is fairly simple: serious failure to keep up with inflation. If the government chooses to do so it may scale up grants for inflation using the university costs index; but the index is retrospective—inflation for last year decides this year's scaling up. The index rose 10.7% in 1973 and 29.4% in 1974 and the UGC was faced with two dilemmas: first, that the acceleration in inflation could not be reflected in disbursements during 1974–75 and second, that the government was not even disposed to allow the 10.7% to go through (academic salaries, comprising half of UGC grants, were properly supplemented). The £187.5 million for general purposes was eventually supplemented by £19 million but the shortfall to universities in the year 1974–75 has been £35.4 million. The recent settlement for 1975–76 allows for inflation in 1975, but the £35 million is lost forever

The consequences of present shortfalls are all too obvious—every university tries to maintain its teaching standards, so research facilities suffer. There are twelve new university libraries planned, but none as yet has a chance of being funded. There are no plans for new science and technology buildings; few staff are being replaced when they leave; and exacerbating all this is the lack of any planning horizon If universities could not know whether there was worse to come, they would go on freezing hiring, and Sir Fred, wearing his *quondam* vice-chancellor's hat, said he could not blame them.

To all of this the committee lent a sympathetic ear—not that there is much more they can do. Only two suggestions disturbed the gloomy tranquillity. Is there any redundant research going on, asked Mr Anthony Nelson—anything coming to the end of its usefulness? Sir Fred was cagey, certainly not everything was equally useful and equally high powered intellectually, but it was not his job to evaluate. Then would you welcome a new body of scrutineers to pinpoint redundant research? No thanks, there have been enough disturbances recently what with Lord Rothschild's report and the economic crisis: such a body in the past would probably have voted to terminate support for Kipping or Fleming. And then Mr Norman Tebbitt (not for the first time) raised the issue of whether postgraduate students from overseas were paying their way. Difficult, said Sir Fred, and I don't fix the fees, but there was a case for increasing charges. The trouble was that international links often returned intangible benefits.

Round Britain

Ironically the only thing working in the UGC's financial favour at present is the disinclination of young people to go to university. Projections for the year 1975–76 are now that there will only be 270,000 students in universities —36,000 less than had been planned for. That is hardly a saving for which the UGC can be grateful.

● The seven largest contract research organisations in the UK are banding together to form an association (AICRO) which they hope will help them to tap new markets and expand old ones.

Income of AICRO members in 1974

Company	£ million
Electrical Research Association	1.300
Fulmer Research Institute	1.060
Huntingdon Research Centre	4.445
International Research and Development	1.500
Inveresk Research International	0.750
Ricardo Consulting Engineers	2.109
Robertson Research International	2.106

Launching the new association last week, its first president, Mr D. Downs, of Ricardo Consulting Engineers, said that the body would have two principal tasks during its first year of existence. First, it would set out to convince government departments of the advantages of having research done by independent organisations — chiefly value for money and efficiency. The association thinks, for example, that the research requirements' boards set up in government departments in the aftermath of the Rothschild report should be less insular and more prepared to let research contracts on the open-tender principle, as is the case in the USA.

Second, AICRO will be looking hard at opportunities abroad, not only in the USA, where it feels its members can "respond cooperatively" in the face of competition from giants like Battelle, Arthur D. Little and the Mitre Corporation, but also in Europe. AICRO hopes eventually to have permanent outposts in Brussels and Washington to ensure that no opportunities are missed.

Although the association is a 'trade association' and in no sense an amalgamation of the seven companies, the terms under which it has been set up include a provision that *ad hoc* consortia shall be formed from time to time as circumstances demand.

● In his introduction to the 1974 report on research and development carried out by the Ministry of Agriculture, the Chief Scientist, Dr H. C. Pereira, stresses the need for Britain to become more self-sufficient in food.

A vastly improved programme of grassland management is one of the top priorities on the lists of projects which could help to bring about this self-sufficiency. Animal feeding stuffs, imported from abroad, have increased rapidly in price over the past few years, and the idea of feeding British livestock from our own land has now become much more attractive economically. Work which the ministry is supporting, either in its own establishments or through commissions to the Agricultural Research Council (ARC) includes improved management of hill grazing lands and the breeding of varieties of grasses and forage legumes suited to this marginal grazing land.

Advanced techniques of field-wilting and barn drying of hay, and of pressure-dewatering of crops of grasses and legumes before they are dried will be applied to most of the forage harvested rather than, as at present, to only a small proportion under advanced management The need for a drastic change in feeding policies for both beef and dairy cattle is evident, says the report, from the overall food supply data for the UK. Measured on the basis of protein intake, the UK carries a feeding burden almost 4½ times that required for its human population of 56 million.

Dr Pereira also forecasts a 'green revolution' for British cereal growing, after the introduction of the new highly productive soft wheats such as Maris Huntsman, bred at the ARC's Plant Breeding Institute at Cambridge, which has achieved on-the-farm-yields of 4 tons an acre, as opposed to the national average yield of 35 hundredweights an acre. Increased demand for home-grown cereals may result in the spread of cereal growing into the wetter areas of Britain, not ideally suited to these crops. This will bring problems of greater susceptibility to disease, and the assessment of cereal foot and root rots—a relatively little worked field of research—has been proposed by the ministry's Plant Pathology Laboratory.

correspondence

Evolving evolution

SIR,—A science writer who spends his life recording the repeated triumphs of unorthodoxy must complain about the tone of R. A. Crowson's remarks on molecular evolution and anti-Darwinism (*Nature*, **254**, 464; 1975). The simplest fault is the most telling: throughout his attack on Ohta, Crowson refers to "him"—but Ohta is a woman. That is a symptom of the lack of ordinary personal communication between the "neutralists" and "selectionists".

There is a lack of scientific communication, too. Crowson makes much of alleged discrepancies between the fossil record and the dates used by Dickerson and by Wilson *et al.* in their molecular chronologies. As Dickerson himself pointed out forcibly: "The zoologists who have the best command of information on dates have maintained a reserved scepticism towards the entire protein endeavour."

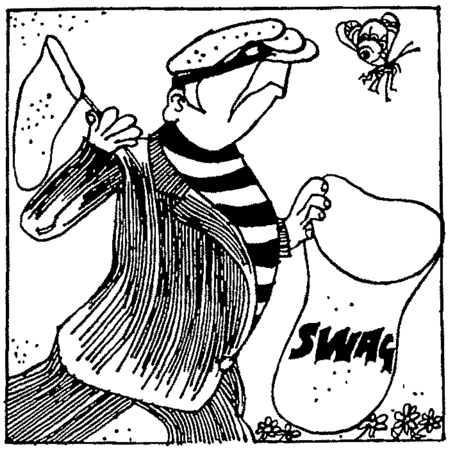
The language Crowson uses is improper for a scientific argument. Concerning his quotation on "the demons of the underworld, chance and probability", one wonders where physicists would be today had they shunned those demons and looked only to "the heavenly powers"? Most perturbing is his phrase "potentially dangerous to our science", applied to the work of Kimura, Ohta and others. Dangerous to whom? To a biological Establishment unwilling to rewrite its lecture notes? If science does not live dangerously, open always to rebuttal and revision, it quickly ceases to be science.

Neo-Darwinism has shown signs of hardening into quasi-religious dogma of a kind that Darwin would surely have repudiated; witness Crowson's phrase about "the historic truths of evolution". Fortunately, discoveries and ideas have kept the blood circulating, not only the puzzling intraspecific and interspecific variations in molecular structure but, in a quite different direction, the violation of the canon against group selection in explanations of social behaviour.

I don't know whether the "neutralists" are correct or not, but then no one does. What I do observe is that some of the critics of Kimura and Ohta react like priests scenting blasphemy. Which really is "dangerous to our science": bold hypotheses which eventually can be thrown out if wrong, or theories too sacred to question?

NIGEL CALDER

Crawley, Sussex, UK



Butterflies lost

SIR,—In 1974 about 15 butterflies were stolen from the Museum National d'Histoire Naturelle in Paris. All the specimens were rare and beautiful *Ornithoptera* and *Papilio*. The museum is understandably anxious to trace their whereabouts and puts their value at 30,000 francs (about £3,000).

Butterflies, it seems, are nowadays treated like postage stamps. Rare and beautiful tropical species are in great demand, and on a world basis the buying and selling (and stealing) of specimens is a growth industry. There are big dealers in many countries, particularly in the United States and Japan but also in Britain, and there are numerous smaller dealers and 'contacts' in tropical countries. For many years Taiwan has been at the centre of the trade, and business in Brazil is said to amount to 50 million specimens a year. The demand is almost exclusively for large and showy species. The wings are often made into jewellery (cufflinks, brooches and rings) or are used to decorate trays.

Hardly anything is known of the size of butterfly populations or if species are endangered by massive exploitation for profit; few countries have laws that restrict the import and export of specimens. Years ago the birds of paradise and other beautiful and exotic birds were brought to the verge of extinction by collectors and dealers, but legislation was eventually introduced and it is not easy nowadays to trade in them. Must we wait until some of the most beautiful creatures on Earth are nearly extinct before we take steps to stop the trade?

D. F. OWEN

Leicester, UK

Nitrogen fixation

SIR,—Current biochemical and genetic attempts to develop nitrogen-fixing bacteria symbiotic with plants now lacking them (some described by John Postgate, *Nature*, January 31, p. 305) raise a significant question.

Sir Peter Medawar has said that when developing a new technology, one should try to imagine the consequences if one succeeded beyond one's wildest dreams. Suppose we developed efficient, enthusiastic, and promiscuous nitrogen-fixing bacteria which, rather than confining their attention to a few agricultural crops in specific areas, became widespread in distribution and in effect on the planetary nitrogen cycle. What then?

Those of us who respect the extensive design experience reflected in present arrangements for fixing nitrogen—even more than we respect man's ingenuity in altering them—might rephrase the question more simply. If some plants are not now leguminous might there not be a reason for it? If there were a better kind of nitrogen cycle, would it not already be here?

AMORY B. LOVINS

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Citation

SIR,—Cawkell (April 3) defends citation analysis largely on the grounds that through repeated co-citation groups of articles come to be "the putative 'core' literature of the subject".

With my youthful idealisms now los in the mists of time I regard repeated co-citations with suspicion if not with cynicism. Here I find myself in sympathy with E. Chargaff (*Nature*, **248** 776–779; 1974). He expressed the view that bibliographies were once comparatively honest but that there is a growing tendency for groups of references to be 'lifted' from one publication to the next. The growth of the scientific literature is such that it is difficult for an author to be fully conversant with the content of all articles that may have a bearing on his work. The citing o papers that have not been read cannot however, be condoned; it leads not only to spurious 'cores' (well exemplified in my own field of study) but also to spurious reputations. A citation should imply an author's personal opinion on the relevance of the cited publication.

E. F. HARTREE

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news and views

'Enigma Variations' of mammalian messenger RNA

from Beverly Griffin

THE significance of the sequences of the 3′-end of messenger RNAs in mammalian cells has for the past few years been a problem for cell biologists. Most messenger RNAs isolated from the cytoplasm of eukaryotic cells have been found to contain polyadenylic acid sequences of varying lengths at their 3′-ends. Attempted explanations for the function of the 3′-terminal poly (A) residues have had to take into account the fact that although most eukaryotic messenger RNAs contain them, not all do (*Nature*, **252**, 268; 1974). Now, before the significance of the unusual nature of the 3′-end of many messenger RNAs has been clarified, attention has been re-directed to the 5′-ends of these molecules by the discovery that they also have unusual nucleotide sequences.

Rottman, Shatkin and Perry (*Cell*, **3**, 197; 1974) described a nucleotide sequence at the 5′-terminus of a wide variety of messenger RNAs, which consisted of 7-methylguanosine linked through its 5′-hydroxyl group *via* a tri-(or pyro)-phosphate group to the 5′-hydroxyl of an O-methylated nucleoside. Although these authors gave a chemically incorrect structure to 7-methylguanosine, it seems nonetheless that their general ideas about the structure of the 5′-ends of eukaryotic messenger RNAs may be correct. A similar kind of structure at the 5′-ends of several low molecular weight nuclear RNAs of Novikoff hepatoma ascites cells had also been reported in an abstract by Ro-Choi *et al.* (*Fedn Proc*, **33**, 1548; 1974). Between them, these two reports have stimulated a great deal of research.

Three papers in this issue of *Nature* by Muthukrishnan, Both, Furuichi and Shakin (**255**, 33; 1975), by Abraham, Rhodes and Banerjee (**255**, 37; 1975) and by Adams and Cory (**255**, 28; 1975) discuss the blocked (or capped) 5′-ends of messenger RNA from two viruses (reovirus and vesicular stomatitis virus (VS)) and from rabbit reticulocyte and mouse myeloma cells In all three papers, 7-methylguanosine has been shown to be the nucleoside used by the cell as a block for the 5′-tri-(or pyro)-phosphate end of the messenger RNA. This is linked to an O-methyl nucleoside which may apparently be one of any of the four bases. It is postulated here (as in the transfer RNAs) that O-methylation may serve to protect the phosphodiester link of messenger RNA from the nuclease activities in the cell, with the protected nucleoside possibly playing an important rôle during the processing of the messenger. Similar results are now appearing, or will shortly appear, in the literature about the 5′-ends of other messenger RNAs, not only from viruses whose genomic and messenger information reside in the same molecules (reovirus, Furuichi *et al*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 362, 742; 1975, and silkworm cytoplasmic polyhedrosis virus, Furuichi and Miura, *Nature*, **253**, 374; 1975), but also in viruses containing DNA as their genomes (vaccinia virus, Wei and Moss, *Proc. natn Acad. Sci. U.S.A.*, **72**, 318; 1975 and Urushibara *et al.*, *FEBS Lett.*, **49**, 385; 1975, Simian virus 40, Lavi and Shatkin, *Fedn Proc.*, **34**, 526; 1975, and adenovirus, see Muthukrishnan *et al.*), and in uninfected cells (including mouse L cells, monkey BSC-1 cells and HeLa cells, see Muthukrishnan *et al.*) The weight of the evidence suggests in fact that methylated blocked 5′-termini may prove to be a general feature of eukaryotic messenger RNAs.

Thus, before a biological explanation has been found for the nature of the 3′-end of mammalian messenger RNAs, a biological explanation must also be sought for the nature of the 5′-end. Some progress has already been made on the rôle of the structure at the 5′-end, notably by the group at the Roche Institute of Molecular Biology (Muthukrishnan *et al.*, page 33, and Both, Banerjee and Shatkin, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1189; 1975). They show first that methylated reovirus and VSV messenger RNAs stimulate protein synthesis (in wheat germ or mouse L cell protein synthesising systems) to a greater extent than unmethylated messenger RNAs. They also show that messengers synthesised *in vitro* in the presence of S-adenosylmethionine by virion-associated RNA polymerases contain the 5′-terminal structures found *in vivo*, and moreover that the *in vitro* synthesised messengers are translated with fidelity in the protein synthesising system. Their conclusions from these, and studies carried out with aurintricarboxylic acid (which inhibits polypeptide chain initiation) and sparsomysin (which inhibits chain elongation), are that methylation (at least with reovirus and VSV) is required for translation and probably occurs at the initiation step of protein synthesis. They do not exclude the possibility that 5′-terminal methylation is also important in the processing of eukaryotic messenger RNA.

None of the studies to date offers any explanation for the choice of 7-methylguanosine as the blocking group for the 5′-end of mammalian messenger RNA. The guanosine may be added to the 5′-end of the RNA by guanosine triphosphate, and this may in part explain the need for GTP in protein synthesis. Guanosine may then be methylated at N–7 by the machinery of the cell. (It is noteworthy that all the chemical studies on methylation of nucleosides show the high susceptibility of the 7-position of guanosine to methylation.) 7-methylguanosine, moreover, is a highly polar molecule at every *p*H at which it can be studied, and has never been isolated except in a hydrated state. Thus, this modified base on a messenger RNA would be expected not only to increase the hydrophilic nature of the macromolecule, but also to help it to form stable salt bridges in, for example, a protein-nucleic acid interaction complex.

Finally, it is conceivable that the blue fluorescence associated with 7-methylguanosine at *p*H 7 (Hendler *et al.*, *Biochemistry*, **9**, 4141; 1970) could be used as an assay for locating and purifying messenger RNAs. This marker might prove of value particularly in messengers lacking the 3′-poly (A) end (where the usual affinity chromatography procedures cannot be used for separating messenger from the bulk of other large cellular RNA molecules).

There seems little doubt that the finding of unusual sequences at the 5′-ends of eukaryotic messenger RNAs will prove a considerable stimulus to research, and may prove to be more illuminating than complicating.

Ecology of human parasites

from Robert M. May

ECOLOGICAL aspects of the relation between humans and their various parasites and pathogens is receiving an increasing amount of attention.

David Bradley (in a chapter in *Ecological Stability*, edit. by M. B. Usher and M. H. Williamson; Chapman and Hall, 1974) has recently drawn together some of this theoretical work and field observation. He distinguishes three types of mechanism whereby parasite populations may be maintained. In the first, parasite populations are determined primarily by the transmission phase. Because transmission depends on the varying environment, this makes for widely fluctuating or 'unstable' populations. This first general mechanism has received much attention in the classical parasitology literature; it invites comparison with other animal populations regulated mainly by "density independent" factors. The second type of parasite density regulation is tied to the host population level, which itself depends on that of the parasite (either through mortality, or the development of complete immunity). This density dependent regulatory mechanism makes for a degree of stability in the parasite population, but remains precariously dependent on the size and structure of a particular host community. The third and most stable mechanism involves parasite regulation by the individual hosts. The parasites in effect use the host's immune system to protect themselves against competition, and maintain infection at a steady but sublethal level: if a helminth has a usual transmission rate of 100 worms inoculated into each host, and the host has a means of preventing the worm load rising above 10, then a tenfold decrease or indefinite increase in transmission of parasites will leave the system unaffected.

This is the ultimate in regulation, and is seen to a varying degree in many parasite systems. Work by Smithers (*Br. med. Bull.*, **28**, 49–54; 1972) and others show that, in Rhesus monkeys infected with *Schistosoma mansoni*, adult worms induce antibody production, which kills or inhibits the growth of subsequent young schistosomes. Thus challenge infections are unsuccessful, while the initial infection persists, continuing its egg production. McCullough and Bradley (*Trans. R. Soc. trop. Med. Hyg.*, **67**, 475–490; 1973) and others have gathered epidemiological evidence suggesting that such a mechanism operates in some human populations infected with *Schistosoma haematobium*, the cause of urinary bilharziasis.

These ecological aspects of the way parasites regulate their populations—by transmission, by overall host population levels, or by host individuals—clearly have implications for public health strategies. If transmission is the key link, then a relatively simple strategy of chemical warfare against the intermediate host (mosquitoes, snails, and so on) is likely to succeed. Conversely, parasites which have evolved stable levels of infection with their hosts can remain essentially unaffected by such a strategy. A clear example comes from Lagos in Nigeria, where a vigorous campaign succeeded in reducing the anopheline mosquito to less than 2% of its previous level, but where the effect on human malaria was very slight. Similarly, in a highly buffered system such as the schistosome–human host relation, a reduction in the intermediate host snail population by one or two orders of magnitude could well have little effect on the prevalence of the infection.

Vaccination against malaria provides another example where ecological theory marches with public health data. Cox (*Nature*, **252**, 268; 1974) has summarised medical evidence to the effect that (at least in monkeys) vaccines can induce not only strong protection against the homologous human malaria species, but also a degree of immunity against heterologous species. Joel Cohen (*Q Rev. Biol*, **48**, 467–489, 1973) observed that, to malaria species colonising a human, "each man is an island, John Donne notwithstanding": thus ecological notions as to the dynamics of island colonisation may be relevant. Such theories would predict an interaction among the species of human malaria, and Cohen surveyed a wealth of data in support of this conclusion. Such theoretical and empirical arguments for a degree of heterologous immunity have obvious practical importance: they suggest that a malaria vaccine need not be specific to each of the species, strains or antigenic variants of *Plasmodium* in order to be effective.

Chemistry should broaden the mind

from J. N Lazonby

The Annual Chemical Congress was held at the University of York on April 7–11. The theme of the congress was 'a view towards the 21st century' and the education symposium reported here dealt in particular with problems of innovation.

"WHY talk about overproduction of chemists?" asked Lord James of Rusholme in his lecture entitled: 'Education through Chemistry?'. "Chemistry courses have been associated too much with vocational rather than educational aspects We must make greater claims for chemistry as an intellectual discipline and place less emphasis on industrial applications and vocational training. Undergraduate chemists must cease to think of themselves solely in terms of future chemists." Lord James saw a PhD as a highly valuable stage in the educational process and not as a final commitment to chemistry.

This viewpoint provided a sharp contrast with the previous lecture given by J. Blears (University of Liverpool), who discussed the supply of chemistry graduates in relation to the needs of industry. He pinpointed the coincidence of the expansion of university science departments with the decline in the proportion of the gross national product being spent on research and development. He suggested that the chemical industry is now sufficiently mature that the need is for economic optimisation of production, rather than for innovation. Thus, from this point of view and also in comparison with other countries, such as the USA, it would seem that we are producing enough first degree chemists and possibly over-producing PhDs for the research and development needs of industry.

R O. C. Norman (University of York) pointed out that universities are good at keeping abreast with the development of subject matter, but there is still much to be done to enhance the educational value (as stressed by Lord James) of chemistry courses. Designers of courses must be more conscious of the qualities of mind which ought to be inculcated. Chemistry courses are good at developing numeracy, but do they develop judgement or verbal skills? Chemistry involves judgement, but students are often not allowed to practise judgement until they are doing research. In particular, he thought that the role of practical work at university ought to be examined more closely. In this respect, he welcomed the University of Sussex 'degree by thesis' as an interesting and worthwhile innovation from which much would be learnt.

Professor Norman stressed that a variety of courses must be provided, ranging from those which are suitable for the future professional chemist to those which offer a study of chemistry in a wider context. But he felt that universities ought not to over-react to student demand and introduce many new courses such as environmental sciences as such aspects may, more sensibly, be absorbed within existing science courses.

The general mood of the speakers on this first day of the symposium was that innovation at all levels of chemical education ought to place greater emphasis on the educational value of courses in terms of the intellectual skills and attitudes which they ought to develop. Also a more liberal attitude ought to be encouraged towards the types of future employment for which chemistry graduates are suited and, as B. M. Kingston (University of Edinburgh) pointed out in his talk, the chemical industry must accept the responsibility of steady recruitment. It was felt that such measures would secure the place of chemistry in the educational system.

The highlight of the second day was undoubtedly the lecture given by J. A. Campbell (Harvey Mudd College, California). He spoke about the CHEM Study project, which, with its stress on a practical approach, has been a major innovation in secondary level chemical education in America. His talk marked the inauguration of joint meetings between the Education Division and the Chemical Education Section of the American Chemical Society. In a most entertaining and stimulating lecture, he accounted for the project's national and international success as indicated by the large scale adoption of the materials. Professor Campbell stressed that the CHEM Study had not been expected to solve all the problems and that its main aim had been to cause 'movement' in chemical education at the secondary level, and it is in this respect that the project has been most successful.

Interplanetary dust cloud

from David W. Hughes

ONE of the main problems in understanding the nature of the interplanetary dust cloud is that most of the observations are made on or near the planet Earth which is stuck in the ecliptic plane at 1 AU from the Sun. If all the orbital parameters of the dust particle can be measured these can then be extrapolated backwards in time and with care a spatial distribution of particles can be built up. But the resulting dust cloud models obtained by different researchers differ widely The value of the density, ρ, of the cloud at 1 AU is about 1.5×10^{-22} g cm^{-3}. Its variation with distance from the Sun, r, is under considerable debate. Calculations have produced ρ=constant ($0<r<3.5$ AU), $\rho\propto r^{-1}$, $\rho\propto r^{-1.5}$ and $\rho\propto r^{-2.5}$, ample proof of the present confusion.

Luckily interplanetary space probes have come to the rescue: one easy way out of the dilemma is to measure ρ using detectors on Mariner and Pioneer spacecraft as they travel towards Mercury and Jupiter. Rhee, Berg and Richardson (Goddard Space Flight Center, Greenbelt) report the results in a recent article in *Geophysics Research Letters* (**1**, 345; 1974). They find that the spatial density of particles with mass greater than 10^{-13} g is independent of heliocentric distance between 0.75 and 1.09 AU and that there is no enhancement around 1 AU. This result agrees with the Mariner II and IV data analysed by Alexander, McCracken and Bohn (*Science*, **149**, 1240; 1965) who found ρ constant between 0.72 and 1.56 AU.

Going further out, data from penetration cells on board Pioneer 10 and 11 indicate that the density varies as r^{-1} from 1 AU out to Jupiter's orbit, this experiment however having a mass threshold of 10^{-9} g. Alvarez, Humes, Kinard and O'Neal (NASA Langley Research Center) reported these results at the 1974 COSPAR meeting. They also found a peculiar Kirkwood-type gap in the dust cloud between 1.16 and 1.35. AU and a two order of magnitude increase in the dust density near Jupiter.

Looking at still larger particles (diameter 35 μm to 10 cm) Soberman, Neste and Lichtenfeld (General Electric Space Science Laboratories, Philadelphia) found $\rho\propto r^{-1.7}$ for the smallest to $\rho\propto r^{-3.2}$ for the largest. Using photometers on the same spacecraft (Pioneer 10) the zodiacal light brightness was found to vary as r^{-2} out to 2.25 AU and then to decrease more rapidly (1974 COSPAR meeting).

Obviously the problem is far from simple, with the variation in spatial density being a function of particle size (this is clearly seen when considering asteroidal sized particles at one extreme and solar wind particles at the other). It also seems a shame that Mariner 10 did not carry a particle-detecting experiment on its way to Mercury because extending the data into 0.3 AU would be very useful. The results from the recently launched West German–US solar probe Helios A which goes in this far will be awaited with great interest.

Very deep fault planes?

from Peter J. Smith

ALTHOUGH earthquakes are being studied ever more intensively, the physical mechanism of deep events still remains uncertain. The patterns of first motions and amplitudes of P and S waves radiated from deep shocks suggest strongly that the double-couple component of the source mechanism dominates other components, but the proposition that the double-couple results from shear dislocation along a fault plane is less easy to substantiate. Moreover, there are other possibilities to consider. Evison (*Bull. seism. Soc. Am.*, **53**, 873; 1963), for example, suggested that deep earthquakes may be produced by rapid phase transitions; and models involving changes of state throughout a finite volume were later analysed by Knopoff and Randall (*J. geophys. Res.*, **75**, 4957; 1970).

Nevertheless, Fukao (*Bull. Earthq. Res. Inst.*, **48**, 707; 1970) and others came down in favour of the more traditional explanation (in spite of the difficulty of envisaging fault motion at great depth) on the basis of their studies of azimuthal variations of the duration of P and S pulses. Even more direct evidence was obtained by Oike (*Bull. Disast. Prev. Res. Inst., Kyoto Univ.*, **21**, 153; 1971) and others who found that some deep events closely related in both space and time lie along a plane roughly coincident with a nodal plane of the focal mechanism solution for one of the events.

The difficulty in attempting to locate possible deep fault planes by observing the spatial distribution of deep earthquakes is that foci must be determined very accurately. In a new study of deep (~600 km) events in the northern part of the Tonga Island Arc, Billington and Isacks (*Geophys. Res. Lett.*, **2**, 62; 1975) have therefore used the method of Joint Hypocenter Determination (JHD) —a method developed by Dewey in 1971 and based on the assumption that for a set of closely grouped foci the combined station and path correction for each station is a constant. To cut a long story short, the use of this technique has enabled relative locations of foci to be determined with a precision of less than 5 km.

With this accuracy, Billington and Isacks show that in two separate cases the earthquakes within a deep cluster lie along, or parellel to and very close to, one of the nodal planes of a large-magnitude event within the cluster. They therefore suggest that these two planes are, in fact, fault planes and that there are probably other such planes corresponding to other clusters. Be that as it may, the two observed planes are nearly vertical and have linear dimensions of about 40 km, which means that the vertical dimension of the lithospheric slab in this particular deep section of the Tonga Arc must also also be at least 40 km (corresponding to a lithospheric thickness of at least 35 km). For the time being, however, the way in which supposed faults relate to the tectonics of the subducting lithospheric slab remains completely unknown.

The past as the key to the present

from D. H. Tarling

DURING the last month 69 papers and a similar number of discussion contributions have been presented at two meetings concerned with the early history of the Earth and Proterozoic tectonics Many significant and often apparently contradictory views were expressed about this period that covers the first 4,000 million years of the Earth's existence.

One of the main items of agreement seems to have come from the dating of lunar rocks and fly-by studies of the terrestrial planets, as it was generally agreed that the Earth experienced a very rapid segregation of its core during a near-molten phase within some 50–100 Myr of its accretion. There was also general but not unanimous agreement that the cessation of major planetesimal infall some 3,900 million years ago means that only localised tectonic features are like to have resulted from impact phenomena so that the apparent similarity between the lunar mare basalts, all younger than 3,100 Myr, and the terrestrial Archaean greenstone belts, some 2,700 Myr old, is fortuitous.

But points of significant dispute immediately arose even from consideration of the accretion process. If the accretion was of an essentially homogeneous mixture, then the earliest core–mantle–crust composition would be geochemically controlled, but inhomogeneous accretion, with predominantly iron-rich material at first, followed by more chrondritic materials, could mean that the composition of the upper mantle was modified after the primary segregation had taken place. From there on the story gets even more interwoven with the inevitable problems of isolating geological facts from interpretations, sorting out semantic problems such as what is understood by plate tectonic processes, and the lack of understanding between different specialisations even when tackling similar problems. These are, of course, the main reasons for meetings such as the very successful NATO Advanced Study Institute at Leicester (April 5–11) and the discussion meeting at the Royal Society (March 13 and 14).

One of the most critical papers for understanding Archaean tectonic processes (before 2,500 million years) was by S. Moorbath (University of Oxford) who demonstrated, in his usual thorough manner, that a very large proportion of strontium and lead isotopes in both granite and volcanic rocks of this age could not have originated by the reworking or partial melting of significantly older sialic crust but must have been derived from the upper mantle. Thus Archaean greenstones and granitic gneisses must have been derived by at least a two-stage process from mantle sources and the metamorphism of the gneisses must have occurred during their emplacement or certainly not more than 50–100 million years later. This evidence will take some time to evaluate, but it clearly means that the Archaean crusts must have been at least comparable in thickness to the present continental crust and that some tectonic process similar to modern plate tectonics probably existed at this time.

The possibility of applying plate tectonic models during this early period was mentioned by several speakers, some for and some against, but the inevitably higher heat flow due to radiogenic heat production must have strongly enhanced convective motions at this time and the higher thermal gradiants would also mean that subduction zones, although more active, would be shallow, possibly accounting for the absence, compared with Phanerozoic times, of andesites (other than those that now seem to be mechanical mixtures of basalts and andacites rather than geochemical differentiates). Nonetheless, there are many features that seem difficult to reconcile with current plate tectonic models; for instance the greenstone belts comprise some 7–10 km of volcanics, usually with interbedded sediments and divded in some areas into two distinct suites. Such belts, each originally covering some 1,500 km,

Elusive hepatitis A virus identified

from Arie J Zuckerman

THE discovery of a specific antigen associated with hepatitis B virus infection has heralded an era of unprecedented progress in our knowledge of this type of infection and in the pathogenesis of liver disease in general. Recent advances with epidemic hepatitis (*Nature*, **252**, 193; 1974), now referred to as hepatitis A, indicate that there may soon be an answer to this hitherto insoluble problem.

The pioneering work of Deinhardt and his colleagues on the transmission of human hepatitis A to species of marmosets (*J. exp. Med.*, **125**, 673; 1967 and *Nature*, **243**, 419; 1973) were confirmed by painstaking experiments conducted over a period of five years by Mascoli *et al.* (*Proc. Soc. exp. Biol. Med.*, **142**, 276; 1973). An agent designated as CR 326 was isolated in Costa Rica from the serum of a child with classical hepatitis A. This virus was passaged serially in marmosets and it was concluded that certain species of marmosets, particularly *Saguinus mystax*, are highly reliable for detecting and propagating human hepatitis A virus. This work was extended by Provost *et al.* (*Proc. Soc. exp. Biol. Med.*, **142**, 1257; 1973) who found that all patients with hepatitis A infection developed antibody which neutralised the CR 326 enterovirus-like agent, but there was no such antibody response in patients with hepatitis B.

In the same year, Feinstone and colleagues (*Science*, **182**, 1026; 1973) demonstrated by immune electron microscopy virus-like particles measuring 27 nm in diameter in faecal extracts obtained from two out of four adult volunteers who were infected with the MS-1 strain of hepatitis A virus (see also *Nature*, **252**, 193; 1974). Similar observations have now been reported on natural hepatitis A by other groups from Australia (Locarnini *et al.*, *Intervirology*, **4**, 110; 1974) and from Arizona (Gravelle *et al.*, *J. infect. Dis.*, **131**, 167; 1975). But perhaps the most exciting reports have emanated from the laboratories of Hilleman and colleagues who developed specific serological tests for hepatitis A antibody using the CR 326 virus antigen. Hilleman presented data at symposia held recently in Milan and in Washington on complement fixation and immune adherence tests. Patients with hepatitis A developed complement fixing and immune adherence antibody soon after the onset of the acute illness and the antibody persisted thereafter. Limited seropidemiological studies have shown that most individuals of a low socio-economic level had hepatitis antibodies and these antibodies were detectable for at least seven years after infection. In contrast, most persons of high socio-economic status in an area of low hepatitis A incidence may proceed to adulthood without infection with hepatitis A.

The availability of serological tests for hepatitis A will provide a valuable tool for diagnosis, for epidemiological studies for detection of hepatitis A virus in attempts to propagate the virus in cell cultures, for the identification of susceptible and immune persons, for assay of hepatitis A antibody in batches of human immunoglobulin and ultimately for vaccine development. A new era in hepatitis research has just begun.

could be entire marginal basins obducted on to the existing crust or, if not stratigraphically continuous, they could represent a series of such basins. On the other hand, if they are taken as Archaean oceanic basins, then they must have since been 'underplated' by sialic rocks to place them in their present setting, as the greenstones are obviously cut and distorted by the diapirically rising later granites, and there is a further 30–40 km of continental crust below the greenstones. Bearing these models in mind, more field work is required, particularly to look for the major shortening and thickening of the crust evidenced by the existence of major nappes and thrusts, now identified by M. Coward (University of Leeds) in Botswana and Rhodesia, before the imposition of intense deformation and migmatisation.

At the start of the Proterozoic, about 2,500 million years ago, or possibly within it (roughly 1,800 million years ago) there seems to have been a marked vertical stabilisation of the existing continental crust. From those times, J. V. Watson (Imperial College, London) showed that it becomes easier to identify the different major continental blocks at almost their present elevation, and the old-established Archaean lineations remained as major factors in controlling the location of Phanerozoic continental separations and possibly mineralisation belts. Evidence for the persistence of horizontal crustal movements during these times was provided by palaeomagnetic studies, although, as E. Irving (Department of Energy, Mines and Resources, Ottawa) pointed out, more than 80% of the available data are still from North America. Revision of even these good North American data illustrated how the palaeomagnetic story from the Precambrian is only at a similar stage to that for the Phanerozoic some 20 years ago.

The possibility of obtaining palaeomagnetic data from the ancient Isua ironstones and Ameralik dykes of South-west Greenland, mentioned by W. Fahrig (Geological Survey of Canada), could provide major evidence on the nature of the Earth's core, 3,760 Myr ago and thus provide stringent constraints for the later evolution of the Earth's mantle, for which G. N. Hanson (State University of New York, Stony Brook) briefly presented apparently convincing geochemical data (to appear shortly in *Geology*), not only for the present existence of deep mantle plumes, but also for a gradual change from an initial mantle-wide major circulation system to the present convection within the asthenosphere.

The Isu formation also figured in consideration of the origin of life on this planet as spheroids within it, some 5–30 μm, could indicate the existence of living matter at this time. More reliable evidence of life, stromatolites, were described within the Rhodesian greenstone belt, some 2,800 Myr old, and the argument about the biogenic nature of structures within the 3,375 Myr Onverwacht Group of South Africa continued. It is, however, in South-west Greenland that the oldest rocks in the world are best exposed and field mapping of the Isua area is likely to be completed next year. There seems to be an excellent case for a 'lunar' type project in this area, bringing to bear all the biological, geological, geochemical and geophysical tools available in order to evaluate the nature of the Earth as soon after its inception as possible.

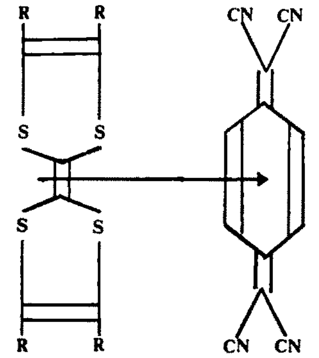
Electronic structure of organic semiconductors

from Andrew Holmes-Siedle

THE cost of semiconductor devices is quite high because, in order to prepare inorganic semiconductors such as silicon or gallium arsenide of the required quality, single crystals must be grown at high temperatures. One of the hopes of manufacturers is that some new material will be developed which can be converted more easily from raw material to finished electronic devices, if possible using a flow process rather than the highly specialised batch processes used at present.

One large range of materials which holds out hope is that of the organic semiconductors. In spite of the normally very poor conductivity of common organic molecules, the fact that organic materials melt, sublime or evaporate at low temperatures and that the raw materials are very cheap have kept physicists working to understand, control and enhance the conductivity of organic media Our good knowledge of the electronic structure of large organic molecules with conjugated double bonds reinforces the hope that the strong local mobility of electrons found within the π orbitals of these systems can in some way be extended over many molecules to endow an organic medium with bulk conductivity.

Progress towards a usable organic semiconductor has been slow over the past ten years (nobody, for example, has yet found a controllable method for forming p–n junctions in organic media). But some interesting prospects remain. One class of materials, the aromatic charge-transfer complexes, such as the complex shown in the figure, while not possessing band conductivity, exhibits very high conductivity (sometimes in the metallic range but more often in the semiconductor range). This occurs by some kind of very efficient hopping process along stacks of the acceptor molecule which have extended wave functions, leading to weak overlap with a neighbour in the stack. Theoretical calculation indicates that several occupied electronic states existing in the tetracyanoquinodimethane molecule are raised considerably in energy by the donation of electrons to this molecule in the charge-transfer complex and that these electrons are the ones which can be transported over large distances (Berlinsky, Carolan and Weiler, *Solid State Commun.*, **15**, 795; 1974).



Tetrathiofulvalene (TTF) **Tetracyano-p-quinodimethane (TCNQ)**

R=H: conductivity 350 $(\Omega\,\mathrm{cm})^{-1}$ (metallic); R=alkyl: conductivity variable but usually semiconducting.

An elegant confirmation of the effect of electron donation on electronic states in TCNQ has recently been demonstrated by Schechtman, Lin and Spicer (*Phys. Rev. Lett.*, **34**, 677; 1975). Previous work had shown that complex formation produced a major effect on the ultraviolet photoelectron spectra (UPS) of TCNQ when alkali metals were reacted with this molecule. The simplicity of the metal ion as donor made the observations less ambiguous than for organic donors. However, since the source of the photoelectron emission is a very thin surface layer, uncontrolled surface impurities could still confuse the result. The new work reduces that possibility by reacting caesium vapour at very low pressure with a freshly sublimed TCNQ film while UPS measurements are made continuously. Before 'caesiation', the TCNQ emission peak is observed at an energy which corresponds to an occupied electronic state at 3.5 eV below the Fermi level. As the caesium vapour reacts, this peak is reduced while two new peaks grow at levels 2.1 and 1.0 eV below the Fermi level. The authors' interpretation is that the 2.1 eV peak is a new position for the state, of symmetry $3b_{1u}$, which was originally at 3.5 eV. They suggest that the 1 eV state is a new 'affinity level', associated with complex formation. These interpretations couple well with the hypothesis of ordered stacks described earlier which, in turn, requires that occupied states must lie very near to the Fermi level so that hopping is frequent.

Interest in the TCNQ series has increased since it was reported (Engler and Patel, *J. Am. chem. Soc.*, **96**, 7376; 1974) that by replacing some S atoms by Se in tetrathiofulvalene, the conductivity of the system can be manipulated conveniently. As far as techniques go, dynamic methods such as the *in situ* UPS method of Spicer's group seem to have the required level of accuracy and directness to throw light on intermolecular bonding in organic compounds. What now remains to be seen is whether intermolecular electron transport parameters can be shown to change dynamically in concert with these measured properties of the individual molecule.

Multi-subunit proteins at Bristol

from John C. Wootton

In a brave attempt to foster communication between crystallographers, biochemists and geneticists working on subunit interactions in proteins, the British Biophysical Society joined with the Molecular Enzymology Group of the Biochemical Society to organise a meeting on 'Multi-subunit Proteins—Structure, Assembly and Control' held at the University of Bristol, March 25–27, 1975.

To what extent do intersubunit interactions stabilise the conformation of individual subunits? Solution studies of the reversible dissociation and deactivation of lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and aldolase were reported by R. Jaenicke (University of Regensberg). Whereas in lactate dehydrogenase inactivating structural changes always seemed to accompany or precede dissociation of the tetramer, various lines of evidence pointed to the occurrence of active isolated subunits of the other two enzymes. All three enzymes will reassociate to the native active tetramer in good yield under conditions which minimise the formation of higher aggregates of incorrectly folded subunits. There was stimulating but inconclusive discussion of how such solution measurements, considered together with intersubunit hybridisation studies, could be interpreted in terms of the number and type of intersubunit bonds known from crystallographic structures. The wealth of high resolution structural information pertaining to subunit interactions and conformational changes in alcohol, malate, lactate and glyceraldehyde-3-phosphate dehydrogenases was reviewed in talks by C.-I. Branden (Agricultural College, Uppsala) and M. G. Rossman (Purdue University).

Direct attacks on the kinetics of subunit assembly and the associated conformational changes were described by K. Kirschner (University of Basel) on the *Escherichia coli* tryptophan synthetase $\alpha_2\beta_2$ tetramer, and by H. Schachman (Berkeley, California), on *E. coli* aspartate transcarbamylase. A pleasing feature of tryptophan synthetase is that activity of isolated α subunits and β_2 dimers can be assayed separately as partial reactions. Assembly to $\alpha_2\beta_2$ is accompanied by conformational changes and increases in substrate affinity and catalytic efficiency. Impressive experimental ingenuity features in both these studies.

Multi-enzyme complexes are well adapted for catalytic efficiency and substrate channelling in situations where free pools of intermediate substrates are undesirable because of instability or interference with other pathways. Multifunctional polypeptides provide a neat means of ensuring a one-to-one ratio of different catalytic activities, and form the basis of a new model for the yeast fatty acid synthetase complex proposed by E. Schweizer (University of Wurzburg). Elegant genetic and biochemical studies of temperature-sensitive fatty acid auxotrophs revealed only two structural genes (which are unlinked (specifying the complex: *fas-1* encoding the dehydration, second reduction, acetyl and malonyl transfer activities, and *fas-2* encoding the condensation, first reduction, and acyl carrier activities. Purified complex dissociated into only two polypeptide chains of molecular weights approximately 185,000 and 175,000. In Schweizer's model the whole complex is built of six repeats of the two-chain, seven-activity structural subunit. This contrasts with Lynen's earlier model in which each structural subunit was assumed to carry a different single activity Interesting genetic criteria were developed which distinguish a multifunctional gene from a gene cluster or an operon· pleiotropic nonsense and frameshift mutants are scattered throughout the fine-structure genetic map, as are pleiotropic, noncomplementing mis-sense mutants (interpreted as defective in folding or assembly of the complex). Evolution of these multifunctional chains from the prokaryotic type of synthetase presumably involved gene fusion.

In the case of the pyruvate and 2-oxoglutarate dehydrogenase complexes of *E. coli* the stoichiometry of the three-component polypeptides is still undecided. R. N. Perham (University of Cambridge) described chemical modification and reconstitution experiments in which a ratio approaching 2 : 1 : 1 of E_1 (decarboxylase): E_2 (lipoyl transacylase): E_3 (lipoamide dehydrogenase) was obtained in the best preparations. But deficiencies of E_1 and E_3 were often observed, and variation in these may account for discrepancies between different laboratories. Perham favours a model based on a 24-subunit 'core' of E_2 with 432 octahedral symmetry, on the surface of which are binding sites (which may be partially unoccupied) for 24 dimers of E_1 and 12 dimers of E_3. Unlike E_1 and E_2, the lipoamide dehydrogenase component is apparently common to and interchangeable between the pyruvate and 2-oxoglutarate dehydrogenase complexes. This is a flavoprotein containing a disulphide bridge which is alternately oxidised and reduced in the catalysis and forms an intrachain six-residue loop. The sequence around this loop is almost identical in the enzymes from *E. coli* and pig heart lipoamide dehydrogenase, and also in yeast glutathione reductase (see Table). It may be a common structural feature of some flavoprotein dehydrogenases, but the four-residue disulphide loop of *E. coli* thioredoxin reductase is not homologous.

Both G. K. Radda (University of Oxford), on the glycogen phosphorylase system, and P. J. Randle (University of Bristol), on the regulatory role of phosphorylation of the mammalian pyruvate dehydrogenase complex, attempted to relate the properties of the purified enzymes to control *in vivo*. Radda described how ^{31}P NMR spectroscopy could be used to estimate the concentrations of free and protein-bound ligands in whole glycogen granules and, in preliminary experiments, in intact rat muscle. Differences between phosphorylase control in the granules and *in vitro* can be explained as resulting from protein-bound metabolites. Solution studies of phosphorylase using spectroscopic probes revealed a multiplicity of ligand-specific conformational changes These are interpreted as locally differing varia-

Sequence around intrachain disulphide loop in three flavoprotein dehydrogenases

E. coli	lipoamide dehydrogenase	-Leu-Gly-Gly-Val-Cys-Leu-Asn-Val-Gly-Cys-Ile-Pro-
Pig heart		-Leu-Gly-Gly-Thr-Cys-Leu-Asn-Val-Gly-Cys-Ile-Pro-
Yeast glutathione reductase		-Leu-Gly-Gly-Thr-Cys-Val-Asn-Val-Gly-Cys-Val-Pro-

tions on only two kinetically significant states. Perhaps many regulatory enzymes, including some of bewildering conformational multiplicity, will prove to be systems of essentially two or a few states.

Naturalists of the past

from a Correspondent

The Society for the Bibliography of Natural History held an Easter Meeting at the Linnean Society on April 3 and 4.

SINCE natural history is a descriptive science firmly rooted in history the researcher in the field has often to consult the older literature and unpublished manuscripts. The four sessions into which the meeting was divided reflected some of the diverse interests of natural history: manuscripts, exploration, descriptive bibliography and social history.

Roy Porter of Churchill College, Cambridge, considered William Hobbs's unpublished *The Earth Generated and Anatomized*, a recent acquisition of the British Museum (Natural History). Practically all that is known about Hobbs is that at one stage of his life, in the late seventeenth or early eighteenth century, he was a government official employed at Weymouth harbour. Although he seems never to have published anything he certainly exchanged ideas with other men of science about the theory of the Earth Opposed to the Woodwardian school that the flood was an agency of fossil distribution, his theories gave a greater prominence to the role of tides in the creation of the Earth. Mr Porter will publish this manuscript after which some reassessment of late seventeenth and early eighteenth century theories of the Earth will be necessary.

The most important purchase made by the Natural History Museum in recent years is the large collection of Sowerby papers. Various members of this talented family played a significant part in the development of British natural history and engraving during the eighteenth and nineteenth centuries by writing or illustrating over one hundred books on botany, zoology, mineralogy and palaeontology. R. J. Cleevely of the Natural History Museum gave a résumé of the material which is as yet an untapped source of information on the production of scientific books in the last century. The Backhouses of Darlington and York, well-known as bankers and nurserymen, were also seminal figures in the growth of natural history studies in the north of England during the eighteenth and nineteenth centuries Colin Simms of the York Museum where the Backhouse collections of plants, bones and bird skins are housed reviewed their work as naturalists. J. Pingree discussed manuscripts at Imperial College, including the papers of T. H. Huxley and Sir A. C. Ramsay.

Michael Hoare of the Australian Academy of Science argued for a more important status in the history of science and letters for Johann Reinhold Forster (1729–98), the controversial naturalist of Captain James Cook's second voyage (1772–75). Phyllis Edwards of the Natural History Museum talked about the botanist, Robert Brown, and the collections of plants, animals and minerals he made during Matthew Flinders's voyage to Australia (1801–05). Peter Whitehead, also of the Natural History Museum, described Piso and Marcgrave's *Historia naturalis Brasiliae* (1648), an important early work on the botany, zoology, medicine and ethnology of Brazil. He is still pursuing his search for the missing original drawings on which the woodcuts in this work were based. Natural history has its share of megalomaniacs and frustrated scientists. G. C. Wallich, naturalist on HMS Bulldog in 1860, became embittered through his vain endeavours to achieve recognition for his scientific work. Antony Rice of the Institute of Oceanographic Sciences in a paper written in conjunction with Dr Burstyn and Dr A. Jones recalled some of the scientific controversies in which Wallich got involved.

When the same plant or animal has been discovered and named independently by different naturalists it is necessary to determine who named it first. Descriptive bibliography, that is the study of books as physical objects, is a discipline frequently called upon to resolve such taxonomic problems by determining dates of publication. John Thackray of the Geological Museum opened the third session with an examination of James Parkinson's *Organic Remains of a Former World* (1804–11) and its various editions and issues. George Washington Sleeper's *Shall we have Common Sense* was found among his papers after his death in 1903. It bore the date 1849 and seemed to anticipate Darwinian theories. A R. Wallace, convinced of its authenticity, communicated it to the Linnean Society and Professor E. B. Poulton made it the subject of his presidential addresses to the Linnean Society in 1913 and 1914. John Collins of Sotheby and Company demonstrated how the application of bibliographical methods proved conclusively that this pamphlet was a forgery. Since illustrations are an integral part of many natural history books it is rather surprising that their biolographical description still remains imprecise. Gavin Bridson, the Linnean Society's Librarian, maintained that a greater knowledge of graphic reproduction processes is required before principles can be formulated for a more exact description of plates in books. Mr Michael Walpole examined textual and plate variations in the editions of William Curtis's *Flora Londinensis*.

David Allen of the Science Research Council traced a pattern of social development in British natural history over the past three hundred years, showing the interaction of natural history and social history. Phillip Lowe of University College, London complemented Mr Allen's thesis with a review of the relationship between amateurs and professionals in the development of science, taking as a case history the emergence of ecology as a scientific discipline. M. McNeil of Darwin College, Cambridge concluded the session with a critique of Erasmus Darwin, who formed a link between natural history, medicine and literature.

The rise of palaeobiology

from A. Hallam

MOST geologists still regard palaeontology as the handmaiden of stratigraphy. That fossils are invaluable in the establishment of a relative time scale was recognised by early last century, and concerns as practical in their objectives as oil companies have felt the need to employ a variety of micropalaeontologists to correlate their boreholes. There is more, however, to stratigraphy than correlation and dating. In its broader sense it is concerned with the interpretation of ancient environments and here again fossils are of great utility. A fossil reef coral, for instance, can tell us much about the ambient sea water temperature, salinity and depth.

The term palaeoecology has been widely used to embrace a variety of disciplines aimed at learning more about environments in the past, such as analysis of the processes affecting fossilisable organisms between death and burial, or the application of knowledge of the tolerances of living organisms to their fossil relatives. The goal in both cases is a geological one and in no proper sense could this sort of work be considered to contribute to our further understanding of biology.

If one attends palaeontology courses in the geology departments of most universities one might be forgiven for failing to appreciate the third great value of fossils. They are the only direct evidence we have of the history of life, of the patterns and rates of large-scale evolution. Palaeontology can in fact provide the invaluable time dimension missing from the subject matter of evolutionary geneticists and ecologists. We might even learn something, furthermore, not just about phyletic lineages but about the evolution of certain communities or ecosystems. Partly as a reaction to what they regard as the somewhat arid scholasticism of a subject devoted more to classification than problem solving, but more positively because they have become excited by new biological concepts and wish to apply them to fossils, a number of younger researchers would now prefer to call themselves palaeobiologists.

The term was used explicitly three years ago in a well-received book edited by T. J. M. Schopf and entitled *Models in Paleobiology*, in which a number of authors devoted themselves to setting up and testing models at a variety of levels ranging from individual growth to ecosystems. Schopf is now the joint editor, with his Chicago colleague R. G. Johnson, of a new journal called *Paleobiology*, published by the American Paleontological Society—the first number appeared in March this year. The editors hope to publish original contributions dealing with any aspect of biological palaeontology, including morphology, biogeochemistry, populations, faunal provinces, communities and ecosystems. The emphasis is on biological processes and patterns including speciation, extinction, development of individuals and colonies, natural selection, evolution and patterns of variation, abundance and distribution in space and time.

How does the first number of the journal measure up to this ambitious programme? Five of the articles are concerned with aspects of functional morphology. Gould and Katz give a computer simulation of the spirally arranged facets of a Silurian receptaculitid and discuss the significance of the geometrical insights thereby obtained. Hopson argues persuasively that the prominent cranial crests of hadrosaurian dinosaurs were sexual display structures and Stanley describes experiments undertaken to throw light on what controls the shape of burrowing bivalves. Two other short articles are concerned with the musculature of brachiopods and crinoids.

The remaining four articles deal with more general matters. Schopf, Raup, Gould and Simberloff continue their computer simulation of phylogeny and argue that fossils of more complex morphology may only appear to have evolved more rapidly, and more controversially that rate of morphological change might bear little or no relation to the underlying genomic evolution. With reference to the evolutionary history of the arthropods, Flessa, Powers and Cisne challenge the widely held assumption that generalised organisms have persisted longer than specialised ones. Raup writes a methodological essay on taxonomic survivorship curves and Levinton and Bambach compare a Silurian with a modern bivalve community. The quality of the papers, in terms of data, thought and presentation, is almost uniformly high.

All the authors of the first issue of *Paleobiology* and the whole editorial board are American. This reflects the fact that the revitalisation of an old discipline has taken place almost entirely in America, probably chiefly because of the flexible teaching system which will allow a student interested in fossils to take biology courses and equip himself for this new area of research.

Are X-ray stars and pulsars related?

by John Gribbin

THE juxtaposition of two papers in *Nature* last week emphasises just how close astronomers may be to producing a unified model of two of the most intriguing discoveries of recent years: X-ray stars and pulsars. According to Tademaru and Harrison (**254**, 676; 1975) pulsars may be accelerated to high velocities by asymmetric emission of low frequency electromagnetic radiation. This is a welcome suggestion for the theorists, since although the theories require that pulsars are formed in supernova explosions, and supernovae are thought only to occur in binary systems, only one pulsar out of more than 100 discovered is known to be in a binary system Clearly, for the theories to hold up the pulsars must have some means of escape from the binaries in which they are born, and the Tademaru–Harrison hypothesis is one intriguing possibility.

Clark, Parkinson and Caswell, on the other hand, have been studying Cir X-1, an X-ray source which may well be both a binary and a remnant left over from a supernova explosion (**254**, 674; 1975). They suggest that Cir X-1 is a runaway from the supernova remnant G321−0.3, and that the X-ray source is in a highly elliptical orbit around a companion. Most models of X-ray stars require the presence of a companion, from which matter falls on to the X-ray star itself to produce X-rays by conversion of gravitational energy into radiation. So it seems plausible that they may be produced in supernova explosions which have not unbound the parent binary system.

Cir X-1, however, is almost an intermediate case. If the model of Clark *et al.* is correct, then the ellipticity of the system now seen is so great that the mass transfer, X-ray mechanism switches off for a time around apastron, when the two components are furthest apart, thus mimicking an eclipse. It is very tempting to see an overall pattern emerging from the observations of X-ray stars and pulsars, in which some systems remain tightly bound after the supernova event, producing 'standard' X-ray sources, others become runaway pairs in which the system remains bound but highly elliptical, producing unusual X-ray stars such as Cir X-1, and in still others the neutron star escapes completely, running away on its own to be seen as a pulsar.

There is one further, interesting implication of these ideas, and that concerns the nature of the star left behind when a pulsar does run away from such a binary system. As the companion must have been involved in mass exchange during the lifetime of the binary system, it may well seem to be highly evolved for its mass; in addition, of course, it is likely to have been somewhat disturbed by the supernova event itself. One consequence of the Tademaru–Harrison model, however, is that there is no need to suggest that if the pulsar accelerates away from the sight of the explosion in one direction then the companion must have moved away in the opposite direction, as there would be an energy mechanism for moving the pulsar independently of its erstwhile companion. So a search for odd, highly evolved stars near the centres of known supernova remnants might not prove entirely fruitless, even if there is already good reason to associate a known runaway pulsar with the particular supernova in question.

A hundred years ago

ON LIGHTNING FIGURES

THE letter headed "Struck by Lightning", and signed "D. Pidgeon", contained in NATURE, vol. xi. p. 405, is valuable, and the more so because it is unaccompanied by any theory. Formerly, when ramified marks appeared on the persons of men or animals, they were always referred to some near or distant tree, of which the marks formed "an exact portrait." Thus, in the *Times* of September 10, 1866, is an account of a boy who had taken refuge under a tree during a thunderstorm, having been struck by lightning, and on his body was found "a perfect image of the tree, the fibres, leaves, and branches being represented with photographic accuracy."

From *Nature*, **12**, 9, May 6, 1875

review article

The GARP Atlantic tropical experiment

B. J. Mason*

The Atlantic Tropical Experiment (GATE), the first major observational experiment of the Global Atmospheric Research Programme (GARP) and designed to explore the tropical atmosphere, was successfully completed in September 1974. In what was probably the largest and most complex international scientific experiment ever undertaken, ten nations—Brazil, Canada, France, Federal Republic of Germany, German Democratic Republic, Mexico, Netherlands, USA, UK and USSR—working in close collaboration, contributed 39 specially equipped ships, 13 large research aircraft, several meteorological satellites and some 5,000 personnel to an intensive three-month study of weather systems in the tropical eastern Atlantic Ocean; in addition, some 50 countries in Africa and South America participated by making additional surface and upper-air observations.

METEOROLOGICAL disturbances in the tropics, which behave differently in many respects from those in middle latitudes, play an important role in the circulation and energetics of the atmosphere as a whole. A better understanding of their working is essential not only for the improvement of weather forecasting in the tropics but, unless their influence is taken into account, it may not be possible to predict the weather in middle latitudes for more than a few days ahead. The main task of the GATE project was to study the structure and evolution of these disturbances and to assess the efficacy of cumulus convection in transporting heat and moisture from the tropical oceans into the global circulation.

Study of large scale tropical disturbances

Most tropical rainfall is produced by convective systems which are the principal mechanisms for vertical transport of heat and water vapour, and probably of momentum. Satellite observations show that tropical convective clouds are not randomly distributed but tend to form in large organised clusters 100–1,000 km across. These clusters are associated with the development of large-scale disturbances, such as the intertropical convergence zone (ITCZ), in the equatorial wind field, and with the troughs of the easterly tropospheric waves which originate often over Africa and sometimes over the Indian Ocean, to proceed across the Atlantic. At least three distinct families of tropical wave disturbances have been identified; the easterly waves of wavelength 2,000–4,000 km in the troposphere, mixed Rossby-gravity waves of 8,000–10,000 km wavelength in the lower stratosphere, and a Kelvin wave of planetary scale in the stratosphere. One of the tasks of GATE is to establish the characteristics of these waves over the tropical Atlantic and the African and South American continents in the belt between 10 °S and 20 °N, to determine their origin, structure, development, propagation, their energy sources, momentum and heat transfers, and relate these to the behaviour of the cloud clusters.

The basic observations of the temperature and wind fields on this large 'A' scale were provided by enhanced synoptic upper-air networks over Africa and South America, supplemented by soundings from GATE ships in the Atlantic and by winds obtained from cloud movements as observed from a geostationary satellite. The network of radio-sonde and radio wind-finding land stations and of some 15 dedicated A-scale ships is shown in Fig. 1. The Russian ships used a radar wind-finding technique whereas most of the remainder used a new balloon-borne transceiver the position and movements in space of which were determined by measuring the time differences between signals received from a number of fixed ground stations belonging to the OMEGA and VLF navigational systems, the computations being performed by small computers on board the launching ships.

Cloud clusters

A much finer network of observations was required to resolve the internal structure of the cloud clusters and the effects of these clusters on the circulation in their immediate environment including the production of compensating downdraughts, the momentum, heat and moisture fluxes into and out of the system at various levels, and the total release of latent heat. Satellite images of the clouds with the contrast enhanced to delineate active convective areas, show that the clusters are composed of meso-scale convective units of up to 100 km in diameter which, in turn, contain a number of cumulonimbus cells of 1–10 km diameter. A much more detailed picture is required, however, to produce a useful working model.

Accordingly there was established a special meso or 'B'-scale network of some 15 observing ships in a 800 × 800 km polygon some 1,000 km off the coast of Africa, south-west of Dakar, in a region where satellite photographs led us to expect a high frequency of cloud clusters, perhaps 10–20 during the 3-month period of the experiment. These ships were required to determine winds to an accuracy of ± 1–2 m s^{-1} averaged over vertical depths of 1 km. Several were equipped with C or X-band radar to explore the internal structure of the cloud clusters and obtain quantitative estimates of the precipitation intensity in order to evaluate the release of latent heat.

A full understanding of the behaviour and effects of cloud clusters will not be achieved without consideration of the fluxes of these quantities produced by the meso-scale convective systems on the 10 km C-scale, and even by individual cumulus cells and smaller-scale motions on the 1 km D-scale. Although these could not be measured throughout the volume of a cloud cluster, sample measurements by specially instrumented aircraft of the motion field of the systems, their spectra of turbulence,

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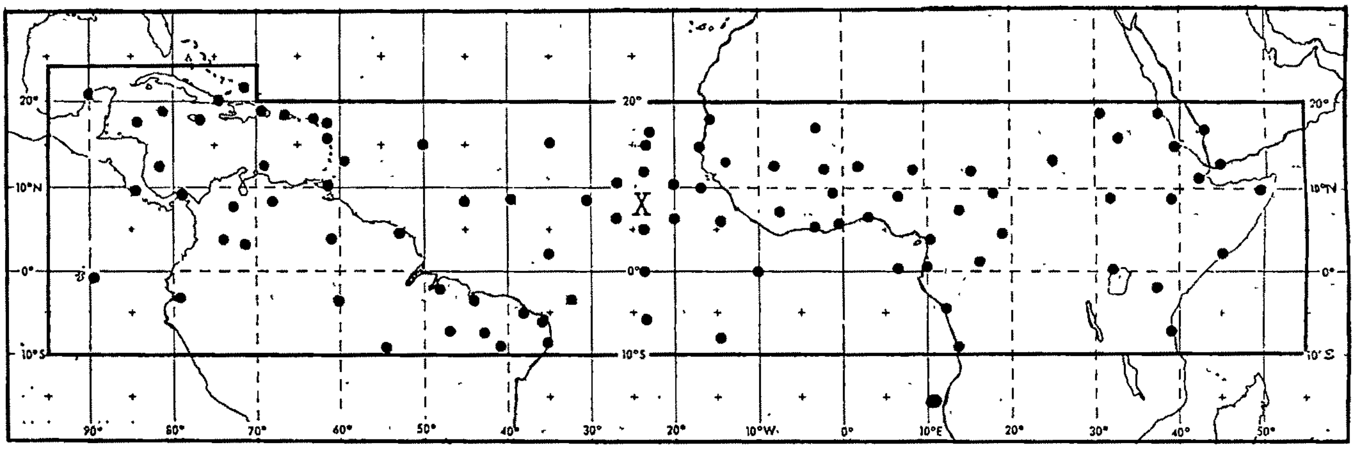


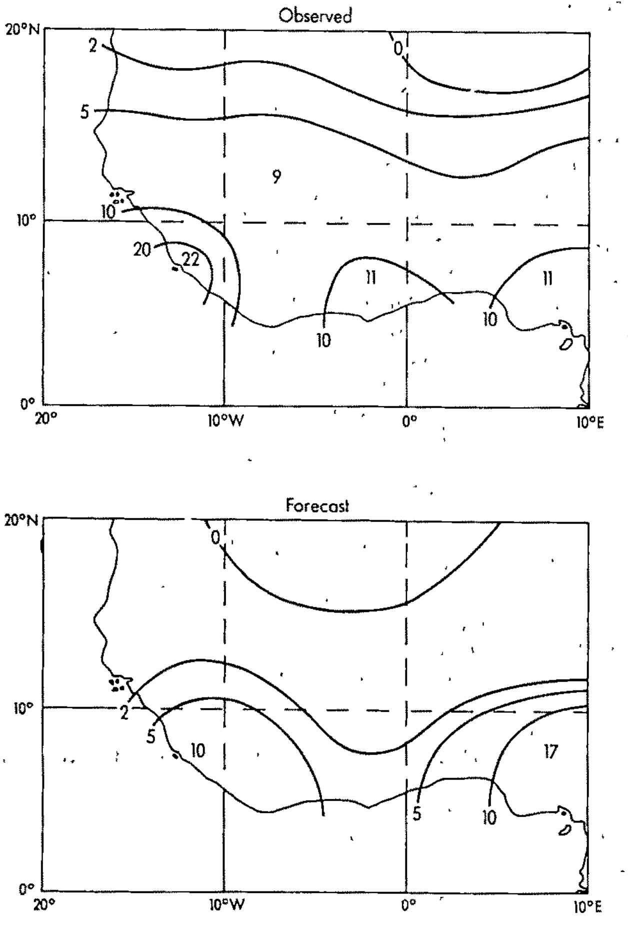
Fig. 1 The distribution of ships and land stations making upper-air soundings in the GATE area. ×, B-scale area.

temperature, liquid-water content and precipitation intensity should provide a valuable indication of their distribution and significance.

Interaction of cloud clusters and larger-scale disturbances

Recent analyses of satellite pictures have revealed that the axes of cloud clusters often coincide with the trough lines of tropical waves and that the fields of divergence, relative vorticity and vertical velocity of persistent clusters coincide quite well with similar fields of the easterly waves. This suggests a close relationship between these systems, with the clusters acting as the rain areas of the wave disturbances. Yet the life times of the clusters (at most a few days and sometimes only several hours) are much shorter than those of the waves (1–3 weeks). Other cloud clusters form in the ITCZ but again their relationships and interactions are obscure and provide an important task for GATE.

Fig. 2 Mean 24-h rainfall (mm) for the period September 4–14 1974 as forecast by the Meteorological Office numerical tropical model compared with the corresponding observed rainfall amounts.



Meanwhile, it has been suggested that convergence in the trade-wind boundary layer produces vertical motions at the top of the layer which, in turn, induce the release of latent heat as a result of the existing conditional instability. The resulting circulation is amplified as the vorticity increases at a rate determined by the linear dimensions of the circulation, the Coriolis parameter and the lapse rate of equivalent potential temperature, and leads at some optimum distance from the equator to the formation of a narrow band of convection—which may be identified with the ITCZ. Alternatively, one may think in terms of the belt of high sea-surface temperature located several degrees from the equator promoting convective activity at these latitudes which, in turn, could excite the development of waves of frequency appropriate to the Coriolis parameter at that latitude. This seems a reasonable explanation for the production of the easterly waves which may then interact with the ITCZ and partially account for its observed variability.

Energy-exchange processes

Whatever the detailed dynamics of these disturbances, which must involve interactions between motions of different scales, their primary role is to link the tropical oceanic heat sources to the global circulation through a series of processes of energy exchange that can be summarised as follows. The absorption and storage of solar energy in the upper layers of the tropical oceans and its transfer to the overlying atmosphere, mainly by turbulent fluxes of water vapour evaporated from the sea surface; continued upward transport of energy through the atmospheric boundary layer by cumulus convection; the release of the accumulated latent heat by condensation in deep-meso-scale convective systems and its distribution to higher levels and over wider areas in precipitating cloud clusters; poleward transport of the heat released in the clusters by the mean meridional Hadley circulation, upper tropospheric waves and standing eddies to feed the global circulation.

These considerations indicated the need to determine the convergence of air in the boundary layer between sea surface and cloud base and to measure the associated fluxes of heat, moisture and momentum, both near the surface and up to cloud base. In disturbed weather, with enhanced cloudy convection, cloud induced mixing may extend below cloud base and couple sea-surface processes with the overlying troposphere. Supporting research programmes to measure at various levels both mean and fluctuating components of the wind, temperature and humidity fields with the required accuracy provided a stringent test of the instrumentation under hostile conditions, a particular problem being with the interference with delicate sensors by radio transmissions from the ships and aircraft. Unique data have none the less been successfully collected on the boundary layer, both by aircraft and tethered balloons and by ships and buoys.

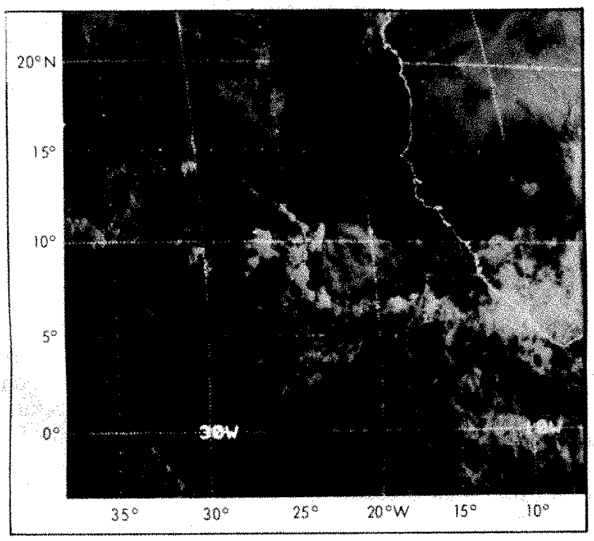
Since long term control of the structure of the tropical atmosphere and its propensity for developing convective disturbances is exerted by the radiative flux divergence, efforts were made in GATE to obtain vertical profiles of the solar and infrared radiative flux divergences and the upward and downward fluxes at both the ocean surface and the tropopause. Measurements at the Earth's surface were made from land and ship stations, but measurements at higher levels required the use of satellites, balloons and aircraft. Since the radiative fluxes were strongly affected by the cloud cover and the water-vapour content of the atmosphere, these quantities were determined independently, mainly from satellite measurements.

The oceanographic experiment

An international group of oceanographers working under the auspices of the ICSU Scientific Committee on Oceanic Research (SCOR) took advantage of the unprecedented concentration of ships assembled for GATE to carry out a parallel programme of observations to obtain independent estimates of the heat, moisture and momentum fluxes across the air–sea interface from measurements in the mixed upper layers of the ocean, and to investigate the response of the oceans to impressed atmospheric disturbances of various scales. Such information will be of great value in developing coupled ocean–atmosphere models for long range predictions of weather and climate.

On the A-scale, detailed studies were made of the structure and dynamics of the cold eastward Equatorial Undercurrent which may have considerable impact on the atmospheric circulation. Measurements showed it to oscillate about the Equator with a wavelength of about 2,500 km, a period of about 18 d and a westward phase speed of about 1.5 m s^{-1}. Measurements of currents, salinity and temperature by the B-scale array of ships will allow the corresponding transports and budgets to be computed. The response of the ocean to smaller scale disturbances such as cumulonimbus with their precipitation and cold downdraughts was followed through their influence on surface and internal waves, vertical current shear, fronts in the thermocline and the transport properties of the mixing layer. To this end, the RRS Discovery of the Institute of Oceanographic Sciences, the Canadian RS Quadra and the US Columbus Iselin towed specially designed probes to make fine-scale measurements of temperature and salinity in the upper 50 m of the ocean and to map horizontal features only a few kilometres across.

Fig. 3 Photograph from the US geostationary satellite poised over the B-scale array showing the cloud cluster which was explored by six aircraft on August 10, 1974.



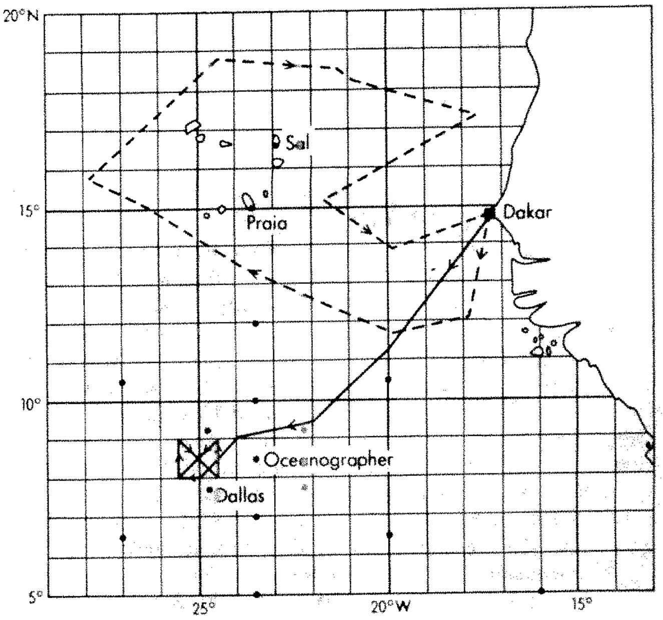


Fig. 4 The 'butterfly' flight pattern followed at several different levels by four research aircraft in exploring the cloud cluster on August 10, 1974 and the flight path (shown dashed) by the high level jet aircraft dropping sondes.

Numerical weather forecasts for the GATE area

During each observing phase of GATE, the Meteorological Office at Bracknell carried out a real-time forecast–analysis cycle updated every 12 h. In each cycle a forecast was produced by an 11-level numerical model developed very quickly for the GATE area and this produced a background field for observations made at the same time; the latter was used to modify the forecast fields and produce the analysis for the next forecast. In this way useful information can be propagated into areas of poor observation, and the best overall analyses obtained. The Meteorological Office experiment had three main objectives.

First, we wished to test new analysis techniques which were designed for the tropics. Most analyses carried out in middle latitudes make extensive use of the geostrophic relationship but in the tropics this is inappropriate. The new methods involved analysing the wind field independently of the pressure field and then adjusting them by a variational technique so that the accelerations implied were not excessive.

The second objective was to test the performance of a numerical forecasting model in the tropics. Numerical models have been shown to give good results in middle latitude conditions, where the main features are cyclones and anticyclones produced as a consequence of baroclinic instability. In the tropics the atmospheric features as large as typical cyclones are relatively weak while small features produced by convective instability are relatively much more important.

Finally, we hoped to carry out the experiment in near real-time to allow monitoring of the number and the quality of observations, to judge which periods should be considered of special interest for later intensive study and to give the personnel a sense of involvement in the GATE experiment as a whole.

The actual execution of the Meteorological Office experiment broadly confirmed previous expectations. It showed, for example, that the parameterisation of convective motions was not always realistic, and in areas of few observations the meteorological situation tended as a result to become dominated by motions on the scale of one or two grid lengths driven by convection. On the other hand in reasonably well observed regions the model did fairly well. It reproduced realistically the structure, development and motion of the westward-moving waves which are characteristic of the GATE area. The rainfall associated with the systems fell into the accepted pattern though amounts were generally less than observed. Figure 2 compares predicted mean 24-h rainfall amounts for the 10-d period September 4–14 with observed values. It shows that, in spite of the scanty observations, the model predicts reasonably well the N–S gradient of rainfall and the maxima near the coast.

Facilities and resources

The network of upper-air sounding stations in the land sectors of the GATE area is shown in Fig. 1 together with the network of special observing ships as arranged for the last phase of the experiment when oceanographic and boundary-layer measurements on the B/C scales were given high priority. All the ships were capable of making routine surface and upper-air meteorological observations and the 19 in the B/C-scale array made a wide variety of special meteorological and oceanographical measurements, 8 of these being equipped with weather radar. Of the 13 research aircraft, 9 were equipped as flying laboratories, a few such as the Meteorological Office Hercules being comprehensively equipped with radar, with an inertial platform, doppler wind-finding equipment and turbulence probes to make accurate measurements of winds and turbulent fluxes of heat and momentum, and with radiometers, cloud-sampling instruments and an onboard data-processing system.

Continual coverage of most of the GATE area by the newly-launched US geostationary satellite was crucial to the whole operation because it enabled weather systems to be kept under continuous surveillance and aircraft to be directed to the most significant of them. Additional coverage was provided by several American polar-orbiting satellites carrying cloud-imaging and temperature-sounding devices.

Most of the ships and aircraft operated from Dakar as a base where excellent harbour, airfield and other facilities were made available by the government of Senegal.

Planning and operational management

It was important to weld the resources into a closely integrated and coordinated international expedition working as a single team. In particular, it was necessary to ensure compatability and comparability between the different national observing systems so that instrumental differences should not mask the properties of the atmosphere that they were designed to measure. This was accomplished by earlier intercomparisons and calibrations of the systems carried by ships and aircraft.

This scientific coordination, together with many other difficult problems of planning, management and logistics, were the main responsibility of the International Scientific and Management Group (ISMG). This international team of scientists, working mainly in Bracknell for the previous three years, produced a detailed scientific and operational plan under the general guidance and instructions of an intergovernmental Tropical Experiment Board that committed resources on behalf of the various governments.

Altogether the experiment had to be carried out with the efficiency and professionalism of a sizeable military operation but with sufficient flexibility to take maximum advantage of the weather and to allow individual scientists and groups to carry out a wide range of experiments with as little mutual interference as possible.

Typical aircraft operation

In this section I describe a typical multi-aircraft mission carried out on one day in the middle of GATE to give some idea of the scale and complexity of such an operation.

From a detailed study of time-lapse pictures from the geostationary satellite (see Fig. 3) and all the relevant meteorological data that led to a forecast of convective activity in the B-scale area, an operation involving six aircraft was mounted on August 10, 1974 to study the structure and environment of a cloud cluster and, at the same time, to provide basic data for the boundary-layer, radiation and oceanographic sub-programmes. A seventh aircraft was used to drop sondes from 40,000 feet and obtain additional vertical profiles of wind and temperature between the B-scale ship array and the African coast.

The Meteorological Office Hercules, carrying the Airborne Mission Scientist and the Control Captain responsible for the coordination of all the aircraft in the exercise, arrived over the B-scale array at 1000. On the basis of satellite information received by this aircraft from the Control Centre in Dakar and radar observations of cloud from the USRS Oceanographer at the centre of the array, the Airborne Mission Scientist selected a suitable cloud cluster for detailed investigation. Three other aircraft, a US DC-6 and C-130, and a French DC-7, then arrived in the area and were joined later by two US jet aircraft, a Sabreliner and a Convair 990.

The first four aircraft flew 'butterfly' patterns at fixed levels between 550 feet and 16,000 feet above sea level, each involving two penetrations of the cloud cluster as shown in Fig. 4. This pattern was flown three times by each aircraft and involved penetration of the active cloud cores. Each aircraft recorded continuously its position, altitude, speed, and so on, the air temperature, humidity, radiation, sea-surface temperature (lower aircraft only) and the wind. Three aircraft carried gust probes to measure turbulent fluxes and a variety of cloud-physics instruments to determine liquid-water content, cloud- and rain-drop sizes, concentrations of condensation and freezing nuclei, and so on. When the radar aboard the aircraft and the USRS Oceanographer showed the cloud cluster to be moving quite rapidly westwards, the flight patterns were modified to allow for this drift. From 1245 to 1320 the Sabreliner made a high level reconnaissance at 39,000 ft above the other aircraft, and the Convair 990 flew the butterfly pattern three times at levels between 30,000 and 35,000 ft between 1300 and 1640. By 1550 the first aircraft was on its way back to Dakar but the last aircraft did not land until 1930 having spent 11 h in the air. Meanwhile, a US jet aircraft had dropped 14 sondes while flying on the track shown in Fig. 4 north of the B-scale area and around the Cape Verde Islands.

Data reduction and analysis

With the field phase of the experiment successfully accomplished, we now face the daunting task of processing and analysing the vast quantity of data collected. Some idea of its magnitude is given by the fact that the UK Hercules aircraft alone recorded some 10^9 words of meteorological data.

A detailed Data Management Plan, aimed at concluding the work within 2–3 years, prescribes in some detail schedules, procedures, methods and formats for the processing, checking, intercomparison and validation of the data and production of the final data sets in the form required by scientists working in the major programme areas and for input to numerical models.

Responsibility for reduction of the observations acquired on national platforms—ships, aircraft, satellites, and so on, lies primarily with the nations themselves but, because of the different systems and instruments employed, these will have to be compared and made consistent using the results of the intercomparison and calibration trials made during the experiment. These tasks of coordination and integration will be carried out at five centres, each being responsible for a particular sub-programme. Coordination between the five centres and monitoring of the whole programme will be carried out by a small group of scientists located in the WMO Secretariat in Geneva. Their task will end in 2–3 years' time with the deposition of the final data sets in the World Data Centres in Washington and Moscow where they will be available on demand to scientists throughout the world.

articles

Mechanistic model for triple junction fracture geometry

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We show that fractures produced in an elastic, homogeneous lithospheric plate by stress generated by wedging of deepseated igneous mass(es) are comparable with the major fracture geometry and rift valley formation associated with the Red Sea, Gulf of Aden and East African Ridges; and that the formation and evolution of certain triple junction fracture geometry (FFR to RRR types) can also be related to stress generated around an oblate magmatic body.

GEOPHYSICAL data[1–4] from the Red Sea and Gulf of Aden indicate the presence of large mafic mass(es) in the central trough(s). In the northern Red Sea, between 17°N and 26°N, the Bouguer gravity anomaly map shows +100 mgal over the centre of the Red Sea with +125 to +150 mgal maximum[4]. This positive gravity anomaly extends over the northern Red Sea south of the Sinai Peninsula and probably as far south as 14°N though the magnitude is much less (Fig. 1). From these data we assumed the presence of a vertically elongated oblate igneous mass at depth extending along the axis of the Red Sea (Fig. 2) and the Gulf of Aden, and analysed the effects of magmatic stress caused by such bodies in producing rifting and fracture geometry (especially FFR to RRR types, where F denotes fault and R denotes ridge[5]) in the elastic lithospheric plates.

Basic data

We used Boussinesq's three-function method as described elsewhere[6–8] to obtain stress distribution(s) around fractures in an infinite and homogeneous elastic rock body. Isolated oblate magma bodies were assumed to exist in isotropic elastic lithospheric rocks in the Red Sea, Gulf of Aden–Carlsberge Ridge and along the East African Rift System. Although idealised, this assumption can be used as a first approximation, if the fractures are propagated essentially in a brittle manner. Because fractures are formed under high stress, the shape of the apex of the oblate ends and the curvature of the upwell or swell and vertical protrusions are considered more important for our theoretical analyses than points, lines or circular holes[9,10]. We analysed stress distributions around vertically elongated oblate igneous bodies of aspect ratios 1 : 10 to 1 : 1000, assuming that the magmatic upwell or igneous mass would continue to grow at depth in areas of asthenospheric plumes[11] or of partial melting. Details are presented elsewhere[7,8,12]. Our analyses show that the absolute magnitude

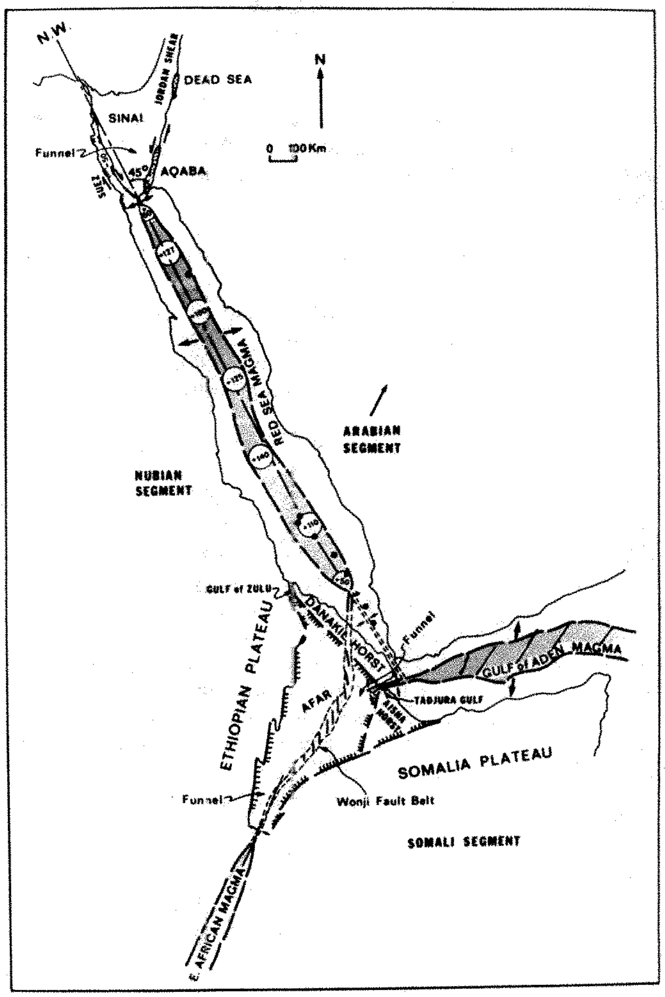

Fig. 1 The Red Sea and Gulfs of Suez, Aqaba and Afar, showing gravity anomalies, major regional stress direction, the principal stress axis due to the Red Sea magmatic source, centres of volcanism (▲) and seismic activity (●). Southern Red Sea showing the probable formation of the Afar depression, *en echelon* normal faulting and graben, and the development of the Wonji fault belt at the axial region of the East African rift due to funnelling or bifurcation of the tensional fracture arm into strike-slip and/or *en echelon* arrays of tensile fracture zone(s) caused by stress from wedging of igneous masses into the lithospheric plate in the Gulf of Aden, Red Sea and East African ridge areas.

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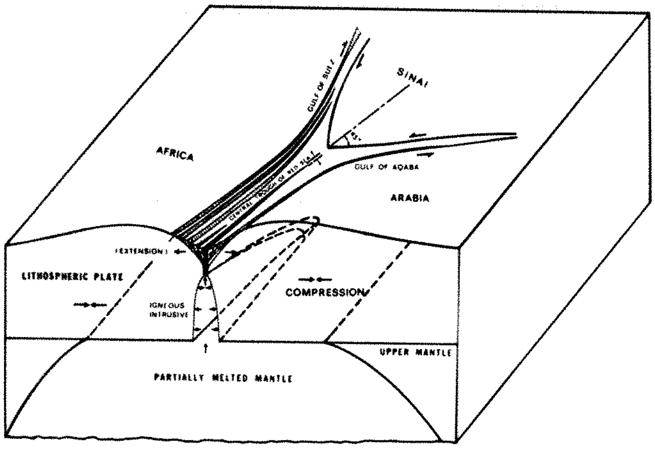

Fig. 2 The mechanism of formation of the central rift in the Red Sea and the FFR type triple junction fracture geometry at the northern end caused by wedging of the dike-like igneous mass into the lithospheric plate. Graben subsidence occurs along a set of funnel-shaped normal faults in the central zone of the rift valley. The areas of compression and extension are shown by arrows; wide domal uplift and relative subsidence in the central region, as deduced from the distribution of elastic displacement vectors are shown.

of the stress contours (Fig. 3) around the apex of an oblate igneous body varies slightly with the change in aspect ratio from 1 : 100 to 1 : 1000. The effect of changing the Poisson ratio of the lithospheric plate from 0.26 to 0.4 was negligible.

These analyses indicate that: (1) elastic stress concentration is greatest around the apex of an oblate shaped igneous body (Fig. 3); and that (2) a vertically elongated oblate body is an ideal shape for the diapiric uprise of magma[13] because wedging of such an igneous mass into the lithospheric plate produces wide areas of lateral compression at the sides at depth but causes horizontal extension within a funnel-shaped zone above the top (Fig. 2).

When magma fluid pressure exceeds lithostatic stress, maximum stress concentration at the apex of the oblate ends may initiate fracture propagation in the elastic lithospheric plate in areas of high stress concentration, along existing lines of weakness or inhomogeneous spots. This initiates FFR type[5] of triple junction fracture geometry (Figs 2–4) with a tensional arm in front of the tip (where later intrusion of magmatic dikes may result in a ridge) branching into two shear zones at an angle of 30–45° with the magmatic stress axis in an homogeneous plate (as the triple junction associated with East Pacific Rise in oceanic plate) and/or *en echelon* arrays of tension fractures associated with or extending along the fracture zones (Figs 2, 3). Although one arm of the shear zone may show transcurrent movement (as left lateral in the Gulf of Aqaba), the other bifurcating arm may show *en echelon* tensile fractures which, with increasing magmatic stress, give rise to continuous normal-faulted zones (the Gulf of Suez, Figs 1, 2). Displacement along the normal-faulted zones may produce subsidence and graben (Fig. 2).

Distribution of elastic displacements above the prolate end of the elongated magma body indicates a wide domal uplift, but an area of relative subsidence and graben would occur in the centre of the domal uplift above the prolate end (Fig. 2) producing an elongated rift valley (central trough) bounded by inward-dipping funnel-shaped normal or block faults as in the Red Sea area[12]. Where magma from deeper levels has wedged or intruded closer to the surface, the fractures (normal faulting) produced by magmatic stress extend upward and outward as in the Red Sea and Rhine graben areas[14,15]. Magmas (mafic or felsic) intruding as axial dikes through such fractures in the central rift zone would produce lateral stretching, further rifting and spreading. Intrusion of a wedge-shaped or slab-like mafic mass through the central normal fault zone would produce areas of relatively higher positive gravity anomaly within the trough and above the rift zone[2,4,14,16].

Our analysis also indicates that stress from broad doming or upwell is not itself great enough to produce axial collapse and rift valley formation of the magnitude of the Red Sea as was proposed by Cloos[17] in 1939. This has also been emphasised recently by Gass and Gibson[18]. Mechanistically, and based on magnetic, seismic and gravity data, the formation of rift valleys and their extension and branching can be explained by dyke-like intrusions of igneous masses into the lithospheric plate below the rift valleys (Fig. 2).

An estimation of stress concentration around prolate protrusions[6,7] above an oblate magma body shows higher stress concentration with increasing aspect ratios from spheroidal to vertically elongated protrusions, and the development of predominantly radial, radial–concentric and concentric fracturing respectively in the lithospheric plate around such pipes, necks or volcanic centres along the axis of the rift valley or normal-faulted zone.

Mechanistic interpretations

The bifurcation of the Red Sea rift at approximately 27°8′N into two shear zones, Gulfs of Suez and Aqaba (Figs 1, 2), the angle of about 45° of the Gulf of Aqaba shear arm with the NW–SE Red Sea axis[4], and the left lateral transcurrent movement of the Gulf of Aqaba–Dead Sea–Jordan shear zone[19–21] are consistent with mechanistic analyses of fracture formation as a result of the Red Sea mafic intrusive mass from the upper mantle (Figs 1–3). Magnetic anomalies and high-level oceanic crust are not present in the northern part of the Red Sea (beyond 25°N), but the central trough continues as a definite bathymetric feature northward toward Sinai where it seems to terminate against the left lateral transcurrent Jordan shear[4,18]. Mechanistically, this part represents a tensional arm (Figs 1, 2) not yet filled by oceanic (mafic) materials at the upper level of the Red Sea crust, bifurcating into normal-faulted shear zones south of Sinai (Fig. 2). This triple junction fracture geometry at the northern end of the Red Sea is interpreted as FFR type because it relates to stress at the apex of the vertically elongated igneous mass along the rift in the Red Sea, and not RRR

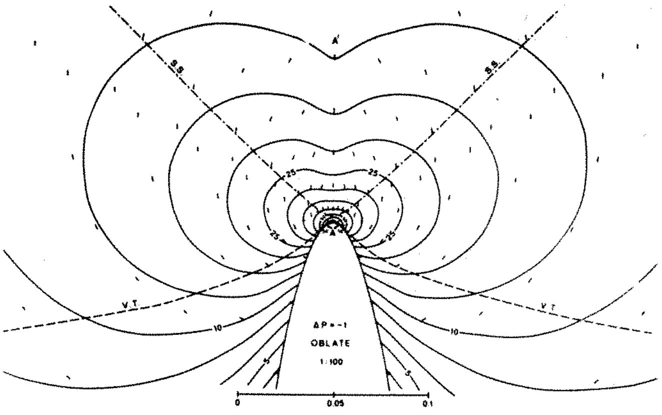

Fig. 3 Contour diagram of maximum tensile stress (in kbar) caused by an excess of magma pressure (P) over lithostatic stress (σ_1) ($P - \sigma_1 = -1$ kbar) around the apex of the dyke-like, oblate intrusive mass. Tensile stress is indicated as positive. Only the contours around the apex (area of maximum stress concentration) are shown. Lithostatic stress should be superimposed on the stress value(s) shown. The direction of maximum stress is perpendicular to the short lines. Above the line VT induced stress due to excess magma pressure is three-dimensionally tensile although absolute tensile stress actually occurs close to the tip of the intrusive mass.

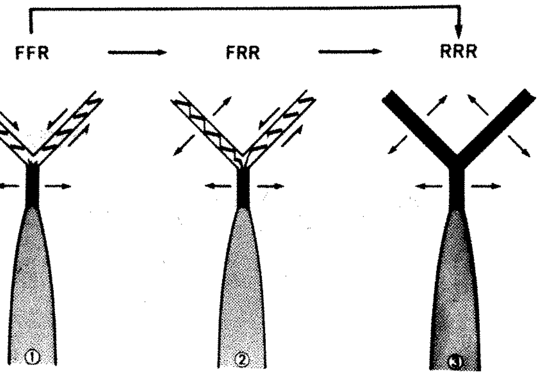

Fig. 4 Development of FFR type triple junction fracture geometry due to an oblate igneous mass and probable path of development of RRR from FFR type triple junction fracture from progressive increase of magmatic wedging and stress.

type with a hot spot at the centre of the triple junction south of Sinai as envisaged by Burke and Dewey[22]. Lack of igneous activity, and large negative gravity anomalies in the Gulfs of Suez (−50 mgal) and Aqaba (−100 mgal) support this interpretation. But continued rifting with increasing magmatic stress and filling of properly oriented fracture arms with magmatic materials can transform an FFR into an RRR type triple junction. This gradual evolution may follow the path FFR→FRR→RRR or proceed directly from FFR to RRR type (Fig. 4). The Gulf of Suez has been suggested to be a "failed arm"[22] of a triple junction; there is no geophysical evidence[4] of its continuation northward in the Mediterranean. On the other hand, the Gulf of Aqaba–Dead Sea–Jordan shear zone is more extensive (600 km) and shows transcurrent movement on the order of 90 to 110 km (refs 4, 20). The 45° orientation of the Gulf of Aqaba–Dead Sea–Jordan shear to the principal stress axis (Figs 1, 2) of the Red Sea igneous mass is ideal for strike-slip movement providing relief of the stress resulting from magma'pressure caused by continued magma generation at depth, rifting, magmatic wedging and spreading along the Red Sea central trough; it adds to the NE movement of the Arabian Plate[23]. This may also account for the low seismicity of the northern Red Sea. Mechanistically, the Gulf of Suez represents an *en echelon* fault zone widened (70–80 km) by normal faulting with continued rifting and increasing stress because of the growth of the Red Sea igneous mass since Miocene. The orientation at 10–15° of the Gulf of Suez to the principal Red Sea axis (Fig. 1) makes it an extensional inactive or "failed" arm of the northern Red Sea triple junction fracture. The orientation of preexisting lineaments in the basement rocks seems to have controlled the low angle orientation of this fracture zone producing the Gulf of Suez.

At the southern end of the Red Sea, the oceanic crust and centres of volcanism within the Red Sea rift seem to extend to almost 15°N (refs 4, 18; Fig. 1). On the basis of seismicity[23], however, the Red Sea rift has been considered as continuing further south to join with the Gulf of Aden and East African rift forming the boundary of the triple junction plates of Arabia, Nubia and Somalia. Mechanistically, the southern extension of the Red Sea central rift represents a tensional fracture arm, developed from stress resultant from further wedging and intrusion of the Red Sea igneous mass, which bifurcates into two shear-faulted tensional belts: the Gulf of Aden and the main Ethiopian rift (Figs 1 and 2). The Gulf of Aden, identified as a tensional feature[24], involves the separation of the continental blocks of Africa and Arabia and the formation of new oceanic crust in between. The belt of Quaternary tensional faulting occurring along much of the axial portion of the main Ethiopian rift, termed the Wonji fault belt[25], is characterised by a series of *en echelon* fracture swarms[26] and is interpreted by Gibson[27] as open tensional faults resulting from left-lateral shear along the length of the main Ethiopian rift. This and Girdler's view[28] agree with our mechanistic interpretation (Fig. 1) which suggests that the origin of the Wonji fault belt is related to stress from the Red Sea igneous mass. An axial zone of relatively high Bouguer gravity values coincides with the Wonji fault belt and injection of basaltic dikes at shallow depth has been suggested[29]. Thus, mechanistically, the triple junction fracture geometry (FFR) at the southern end of the Red Sea is being transformed to RRR type with magmatic wedging and intrusion in the Gulf of Aden and the main Ethiopian rift.

The Gulf of Tadjura represents a tensional feature (Fig. 1) produced by rifting as a result of the Gulf of Aden's magmatic stress. If its origin is related to this magmatic stress, the Gulf of Tadjura's tensional arm should bifurcate into two *en echelon* normal-faulted tensional or shear zones. These seem to be: one, paralleling the Red Sea along the western Danakil block and extending to the Gulf of Zulu; the other extending along the western margin of the Aisha block toward the Somalia Plateau (Fig. 1). Normal faulting toward Afar along these bifurcating fracture arms would produce the Danakil and Aisha Horsts (Fig. 1). Stress caused by the igneous mass along the East African ridges would cause bifurcation or funnelling into two *en echelon* normal-faulted zones (Fig. 1). Such funnelling of the East African ridges into the Dankelina fault along the Ethiopian side and the East African rift along the Somalia side is well known[30]. Normal faulting along the en echelon tensional zones of Ethiopia, Somalia and Danakil height [29] (Fig. 1) would produce a depression similar to Afar with a high plateau or horst on each side of the triangle. Thus the origin of the Afar depression can be directly correlated with the stress fields generated by the wedging of the Gulf of Aden and the East African igneous masses. At the northern end of the Ethiopian plateau and the Danakil height, the normal-faulted zone(s) narrow into the Gulf of Zulu (Fig. 1) and mark it as a tensional zone. Mechanistically, all the Gulfs (Suez, Tadjura, Zulu, and so on) are tensional features. Recent active rifting, spreading, volcanism and the formation of graben along the tensional zones within the Afar depression[31] may result from complex interaction of stress fields and fracturing in the Afar crust caused by the East African, Red Sea and Gulf of Aden's magmatic stresses.

The analysis also suggests that one need not invoke the presence of hot spots or asthenospheric plumes[22] at the intersection of triple junctions to explain every example of major or minor triple junction (FFR or RRR type) fracture geometry.

This work was partially supported by the NSF. We thank Dean M. Gabriel of Brooklyn College for additional support and computer facilities.

Received October 8, 1974; accepted March 5, 1975.

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Climatic changes, Norsemen and modern man

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Oxygen isotope analysis of a new ice core from the crest of the Greenland ice sheet reveals a climatic record of the past 1,420 years. Climatic changes of medium frequencies are in phase with corresponding changes in Iceland and England, whilst long-term changes at mid Atlantic longitudes are out of phase with Europe and North America. Reconciliation with Norse history suggests a strong climatic impact, and a parallel is drawn to the present critical situation of the human society.

POLAR glaciers contain palaeoclimatic information in the form of varying isotopic composition of the ice, as given by the δ-function, the per mille (‰) deviation of the concentration of the heavy oxygen isotope ^{18}O in ice from that in Standard Mean Ocean Water[1-4]. During the precipitation process, the heavy isotopic component, $H_2{}^{18}O$, condenses preferentially (because its vapour pressure is slightly lower than that of $H_2{}^{16}O$), which causes decreasing δ in the vapour and subsequent precipitation at high latitudes as the cooling proceeds. Consequently, at a given location snow and ice deposited in summers or in warm climatic periods have higher δ than snow and ice deposited in winters or in cold climatic periods.

On this basis, continuous δ profiles along ice cores from the polar ice sheets have been interpreted in terms of seasonal or climatic variations. But the climatic interpretations have hitherto been hampered by a poorly determined relationship between δ and temperature variations. For example, a given long term change in δ along a deep ice core spanning many thousands of years may be partly caused by a change in surface altitude caused by a general climatic change[1-3]. The altitude effect is hardly important as regards intermediate ice cores (⩽400 m) from essentially stable parts of the ice sheet. Nevertheless, even in such cases, the temporal δ/temperature relationship is difficult to demonstrate directly, first because of the lack of reliable temperature records beyond the instrumental epoch, which only spans one century in Greenland, and less in other high polar regions, and secondly because short term climatic changes (periods shorter than 50 yr) are not completely equivalent in all parts of Greenland (Fig. 1).

Figure 1 shows observed temperatures in Godthaab (64°11′N, 51°45′W) on the West coast (heavy curves) and in Angmagssalik (65°36′N, 37°39′W) on the East coast (thin curves), smoothed by predictive low pass digital filter techniques removing all oscillations shorter than 10 years (to the left) and 30 years (to the right). The smoothing to the end of the record was accomplished by first extending the record using maximum entropy procedures[5]. The low pass filters have sharp frequency cutoff with insignificant influence from the side lobes. Generally, the short term oscillations are not in phase, and differences occur even in the 30-yr smoothed curves; for example the intermediate warming around 1960, only occurred on the West coast. Consequently, temperature records need further smoothing in order to reveal common features of Greenland climatic changes. This also holds for δ records along ice cores and this is why a 60-yr filter has been applied later in this work.

Obtaining the data

In the search for a representative area on the ice sheet, three drillings to 400 m have been made since 1971 by the Greenland Ice Sheet Program (GISP), an international joint effort with contributions by the USA, Denmark and Switzerland. The ice cores were recovered by the thermal drilling technique, and they are being studied by various analytical methods[6].

The first two GISP cores were recovered from DYE 3 in South Greenland (65°11′N, 43°50′W) and Milcent in central

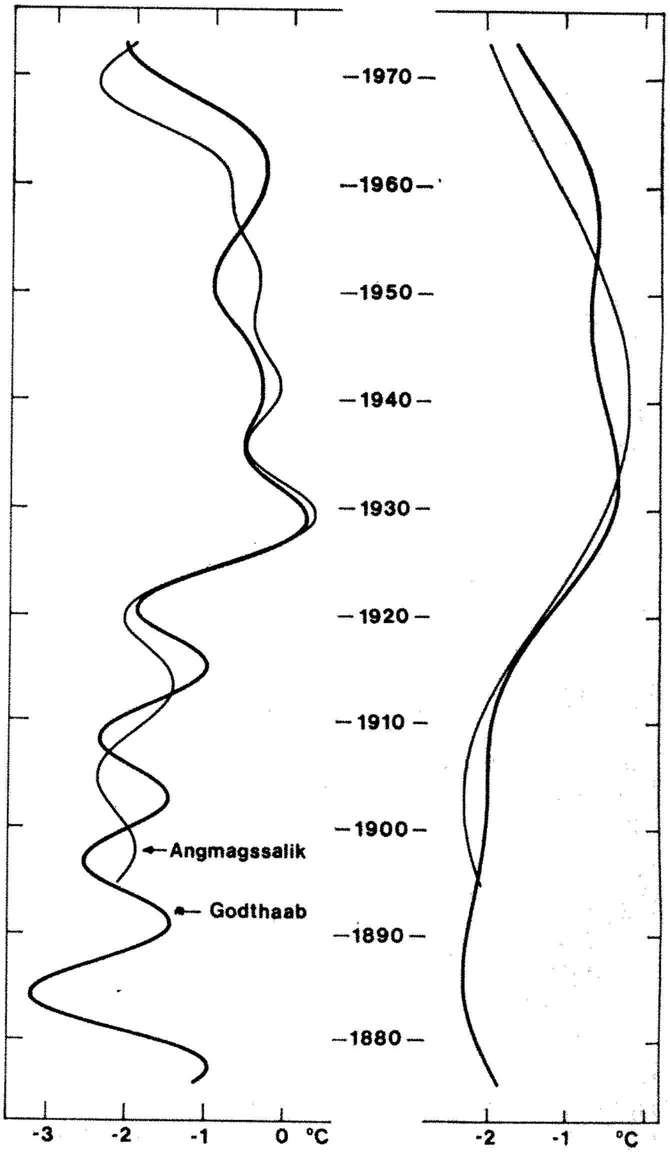

Fig. 1 Observed Greenland temperatures smoothed by 10-yr (left) and 30-yr (right) low pass digital filters. High frequency climatic changes do not always occur simultaneously on the West coast (Godthaab) and on the East coast (Angmagssalik).

Greenland (70°18′N, 44°35′W). Seasonal variations in δ were measured in a continuous sequence over the entire core length for the purposes of dating and accumulation rate measurement. Counting δ summer maxima downward from the surface leads to time scales along the cores that probably deviate less than 5% in the upper layers, and hardly more than 10 yr from absolute chronology at any depth. The oldest layer in the two cores dates from ~1232 AD and 1176 AD, respectively. The detailed plot of δ as a function of depth also gives the thicknesses of the individual annual layers. After corrections for vertical strain since the time of deposition, and for changing accumulation rate upstream, a smoothed plot of layer thickness as a function of time shows that the accumulation rate has kept surprisingly constant over the nearly eight centuries spanned by the cores[3]. This justifies the assumption made below of essentially constant accumulation rate at other locations in the same part of Greenland. In cases where no seasonal variations of δ have been measured, a time/depth relationship may be established by using the following formula[7] for the age t of an annual layer now situated y metres above the bedrock:

$$t = [(2H-h)/2a]\ln[(2H-h)/(2y-h)] \qquad y>h$$

a being the accumulation rate, H the thickness of the ice sheet, and h the value of y above which the horizontal velocity profile is assumed to be uniform. For values of H of the order of 2,000 m or more, h is not critical for the time scale over the upper 400 m. In the case of the DYE 3 and Milcent cores, application of the formula gives time scales that at any depth deviate less than 10 yr from the chronology obtained from seasonal variations of δ measured along the cores.

A third ice core 404 m long was recovered by GISP in the summer of 1974 at Crête on the crest of the ice sheet, 3,172 m above sea level, in Central Greenland (71°07′N, 37°19′W). A time scale based on seasonal variations of δ involves 16,000 measurements and has not yet been completed.

The ice flow pattern is, however, extremely simple in the Crête area, and the accuracy of the time scale defined by the formula is therefore expected to be even better than at DYE 3 and Milcent. With $H = 3{,}150$ m of ice equivalent[8], $h = 600$ m and $a = 0.287$ m yr^{-1} (measured by seasonal variations of δ in the upper layers and checked by β activity analyses), the formula shows that for $y = 2{,}770$ m, corresponding to the bottom of the bore hole, the core reaches 1,420 yr back in time. Accordingly, 1,420 samples were cut from the ice core, each one representing one calculated year of accumulation. The corresponding δ values (available on request) are of course not applicable for very short term considerations and were smoothed by a 60-yr low pass filter. The resulting record, spanning the time back to 554 AD, is shown in the left section of Fig. 2, with a per mille scale on top.

Temperature variations

The temperature scale for Godthaab, West Greenland, below the curve (Fig. 2) has been calculated by comparing the 0.9‰ δ shift from 1890 to 1935 with the corresponding 2.1 °C change in Godthaab temperatures (observed since 1876 and smoothed by a 60-yr low pass filter). Because of the short period of observation it is difficult to estimate the temperature/δ relationship very accurately, in spite of the correlation coefficient being as high as 0.94. With this reservation, and assuming that the δ curve is also closely related to Godthaab temperatures before 1876 (which it is to Iceland temperatures, see below), the δ curve may be read in terms of Godthaab temperatures at least through the seventeenth century and, most likely, over its entire time span.

The upper part of the mid section of Fig. 2 shows a record (smoothed the same way) of observed and estimated temperatures at Stykkisholmur, Iceland. For the 1590–1846 interval the temperatures were estimated[9] by considering the systematic observations of sea ice reported in the Icelandic annals. These observations were calibrated in terms of temperature by comparison with the Stykkisholmur temperatures through the period 1846–1966. The upper part of the smoothed δ record agrees (0.88 correlation coefficient) with the Iceland temperatures back to 1590 (significant at the $P=0.005$ level

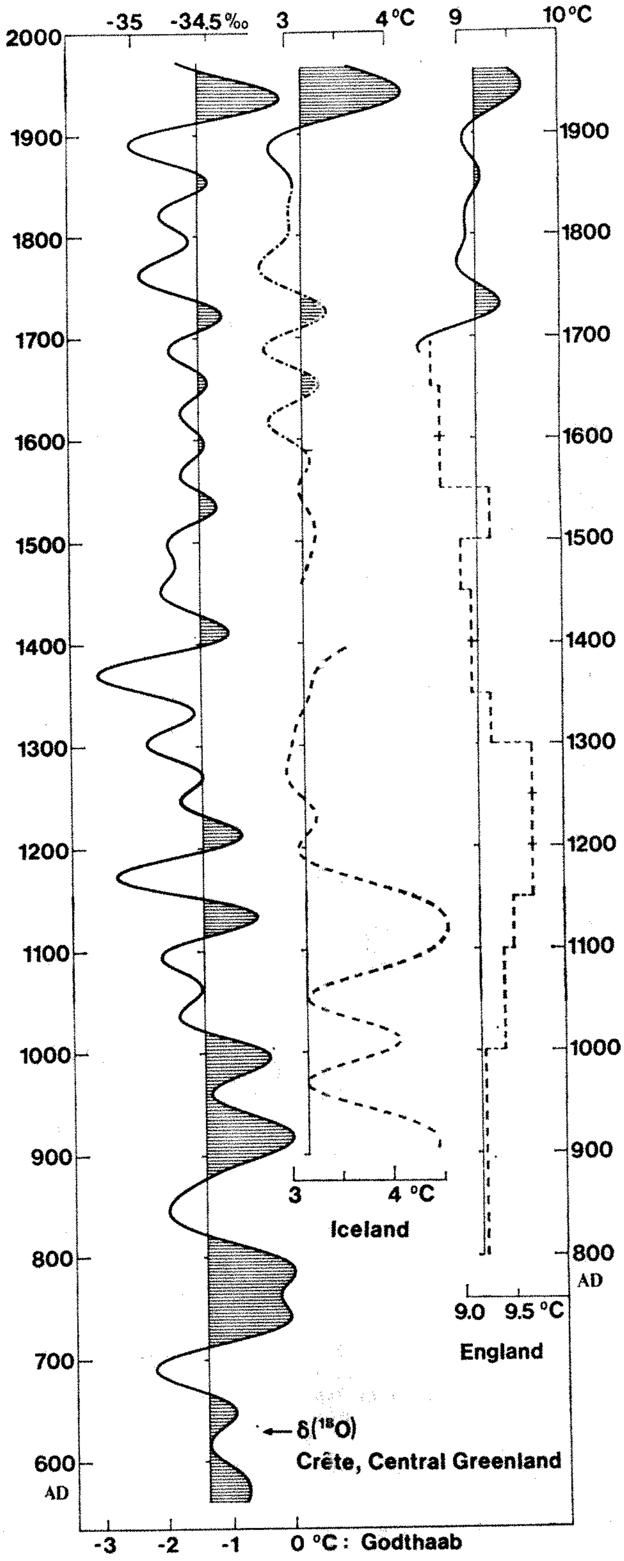

Fig. 2 Comparison between the ^{18}O concentration (left) in snow fallen at Crête, Central Greenland, (δ scale on top), and temperatures for Iceland and England. The curves are smoothed by a 60-yr low pass digital filter, except for England 800–1700 AD. The full curves are based on systematic, direct observations, the dashed-dotted part of the Iceland record is estimated from systematic ice observations[9], whereas the dashed curves depend on indirect evidence[9,14].

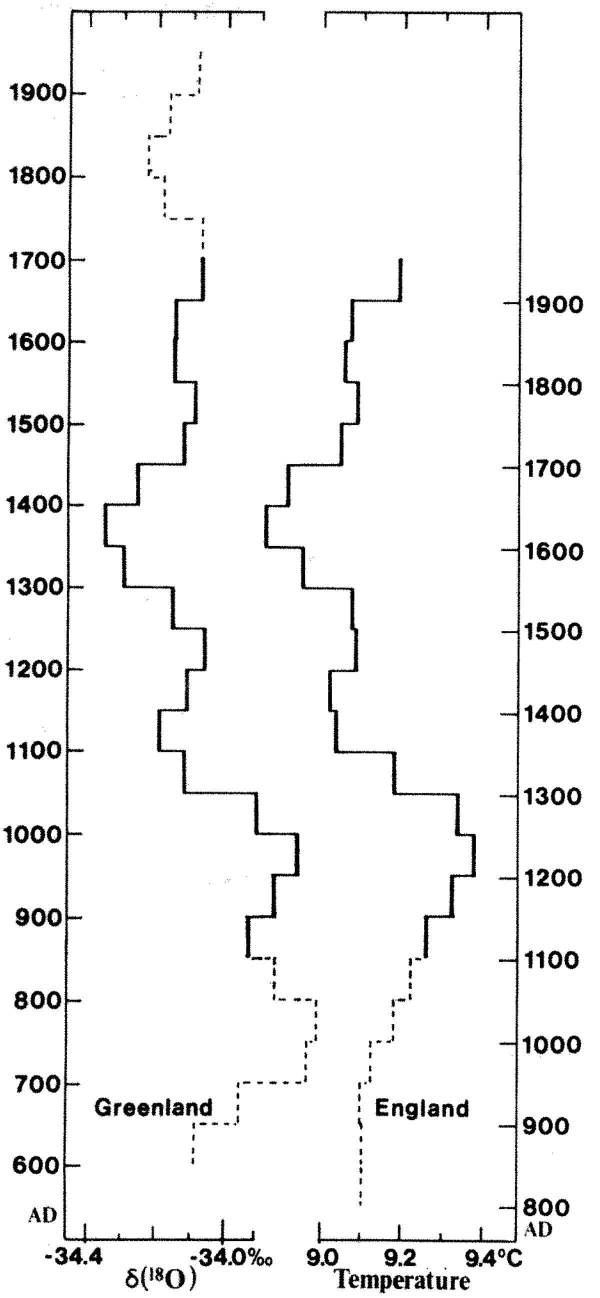

Fig. 3 Correlation since 850 AD between long term trends of 50-yr means of $\delta(^{18}O)$ in Greenland and temperature in England 250 yr later (notice different time scales). The data for England are those shown in Fig. 2, except that all oscillations shorter than 200 yr have been removed by a low pass digital filter. The data for Greenland have been treated accordingly.

with an estimated number of degrees of freedom $n = 7$). The regression coefficient is 1.4 ± 0.3 °C per 1 ‰ change in δ.

In England, temperatures have been measured more or less systematically since 1680 (refs 10, 11). The full curve in the upper right part of Fig. 2 shows this unusually long record smoothed by a 60-yr low pass filter. Comparison with the δ curve gives a correlation coefficient of 0.81 ($P = 0.02$, $n = 6$) and a regression coefficient of 0.8 ± 0.3 °C per 1 ‰ change in δ.

The significant correlation between the upper parts of the curves suggests that the δ curve is representative of climatic changes far beyond the Greenland area. But this conclusion applies only to the climatic changes of medium frequencies (60 to 200 yr periods) that strongly dominate the records through the recent centuries (for short periods, the δ curve is barely representative for the whole of Greenland, see Fig. 1). Changes of lower frequencies that are obvious in the δ curve prior to 1500 AD must be compared with records based on indirect evidence.

Other climatic indicators

In the mid section of Fig. 2 the 60-yr smoothed Iceland curve before 1590 is derived[9] from occasional information in Icelandic annals about sea ice, mild or severe winters, years of good or bad crops, and so on. In spite of the lack of direct meteorological evidence, most of the pronounced medium frequency oscillations back to 900 AD are essentially in phase with the δ curve. The annals[12] tell about very cold years 1367–79, but only little about sea ice. Though not reflected by the dashed curve (Fig. 2), this cold period may be reconciled with the δ-minimum late in the fourteenth century. For the fourteenth century as a whole, there are different opinions among Icelandic historians. Some claim[13] that it was colder than the thirteenth century. Very little information is available from 1400–1470, when the country was afflicted by famines and bubonic plague; but the essential agreement between several details in the two curves is an interesting consequence of the inveterate literary tradition in the early Icelandic society, culminating with the sagas that were written about 1100–1300 AD.

A similar extension of the temperature curve for England is shown in steps before 1700 AD to the right in Fig. 2. It is estimated from various kinds of historical information of meteorological significance[14]. In the 1100–1700 AD interval each step represents 50 yr, and before 1100 AD only two estimates are given; one for the eleventh century and another for the preceeding two centuries. Therefore, the curve only allows considerations of long term climatic trends (periods longer than 200 yr).

The twelfth and thirteenth centuries were extremely warm in England. This must be considered a well established fact, especially since it is substantiated by palaeobotanical evidence; for example, according to ref. 14 vines were grown commercially in England from 1000 to 1300 AD (which suggests even higher temperatures than the more direct meteorological evidence). In fact, the period of extreme warmth may have begun earlier, since there were several vineyards in England before 1000 (H. H. Lamb, personal communication). Thus, the time of the onset of the mediaeval warmth is more uncertain than the time of its termination, which took place around 1300 AD, 100–150 yr later than in Iceland at longitude 20°W, and 250–300 yr later than at Crête at about 36°W.

Furthermore, it is striking that the cold period from 1450 to 1700, often called "the little ice age", was not particularly cold at Crête or in Iceland. The Crête counterpart may be found some 300 yr earlier, from about 1150 to 1400. Nor does "the little ice age" of 1450–1700 stand out clearly in the Milcent (44°W) δ profile. On the other hand, it appears in δ profiles further West at Camp Century[1] (61°W) and in tree-ring records from the White Mountains, North America[15] (119°W). In the latter profile the mediaeval warmth also appears in phase with Europe.

So long term climatic changes at mid-Atlantic longitudes seem to be out of phase with similar changes in Europe and in North America. The phase shift between Crête and England is further illustrated in Fig. 3 showing sequences of 50-yr means smoothed by a 200-yr low pass filter. Apart from the relatively uncertain lower part of the England curve, the variation in temperatures in England from 1100 to 1950 are parallel to the 850–1700 part of the Crête δ curve with a correlation coefficient of 0.92 ($P = 0.01$, $n = 6$). Cross spectra show essentially the same 250-yr time lag and a very high coherence for all oscillations longer than 200 yr. This shows that, within the last millenium, long-term climatic changes have occurred 250 yr later in West Europe than in Greenland.

The data do not, however, enable any conclusion to be drawn about the persistency of the time lag over long spans of time because the high value of n used above, and the consequent high confidence level, is chiefly a result of a cycle close to 250 yr in each series. This cycle is in phase in the cross spectrum of the synchronous series, and will, of course, be so in any cross spectrum of the series displaced by multiples of 250 yr. If the time series in Fig. 3 were smoothed by a 400-yr low pass filter, the correlation coefficient would still be high (0.99), but not significant ($P = 0.10$, $n = 3$). Thus, the upper, dashed part of the δ curve in Fig. 3 may, at most, suggest a general cooling trend in West Europe within the next century. There are other reasons why caution should be applied in this respect. For example, the actual climatic changes in the past 300 yr have been dominated by oscillations of higher frequencies than those considered in Fig. 3; and man-made pollution of the atmosphere may completely change the picture.

A possible explanation for the delay of the shift from the mediaeval warmth into "the little ice age" may be found by considering the quasi-stationary Rossby waves (disturbances of the general circumpolar westerly winds in the upper troposphere, caused by blocking anticyclones) the position of which determines the meridional wind components. If the average wavelength of the Rossby waves increased (perhaps because of a varying radiation balance), it may have caused a slow eastward displacement of the climatic belts in the Northwest Atlantic region, at the same time as a southward displacement of the polar front may be responsible for the general cooling of the Northern Hemisphere. A changing gross wind pattern would influence the course and the intensity of the Gulf Stream, running in between the two areas considered here, which would enhance the climatic effect.

Norse history

With due consideration of the probable 150 yr time lag between the end of the mediaeval warmth in Greenland and Iceland, it is tempting to try to reconcile the δ curve in Fig. 2 with some well documented important events in the early Icelandic and Norse societies. The Norwegian farmer Floke Vilgerdson made the first attempt to settle in Iceland in about 865 AD, during a short cold period. He lost his cattle in a severe winter and disappointed went back to Norway after having seen "a fjord filled up by sea ice. Therefore, he called the country Iceland" (Landnam Saga, about 1200 AD). Only a few years later, in 874, Ingolf Arnarson succeeded. He was followed by many others, and landnam (settlement) was completed in 930 AD. The δ curve suggests that the landnam was favoured by a considerable and rapid climatic warming late in the ninth century.

In 982, Erik the Red discovered new land West of Iceland. He called it Greenland; according to the Greenlander Saga this was only to persuade people to follow him, when he founded the Østerbygd in South Greenland in 985. But the δ curve suggests that the name described a reality, since Erik came to Greenland at the end of a warm period longer than any that has occurred since. The suspicions of the author of the Greenlander Saga may be explained by the long term climatic deterioration reaching Iceland 150 yr later than Greenland.

So, the drastic climatic change late in the ninth century may be part of the reason why Iceland and Greenland did not get the opposite names, which would have been more natural had they been discovered simultaneously.

The cold fourteenth century marked the beginning of the tragic end of the Norse society in Greenland. Shortly before 1350 the Vesterbygd in the Godthaab Fjord died out, and some 100 yr later the Østerbygd as well. The reason is not clear. The early hypothesis about degeneration by endogamy[16] rested on flimsy evidence, because only a few skeletons from the fourteenth and fifteenth centuries have been found; this is in itself peculiar in view of the hundreds of skeletons known from before 1300. A possible reason is that the increasing permafrost in the soil forced the late Vikings to change their burial customs. The δ curve suggests that the climate became so cold in the fourteenth century that years of famine must have occurred frequently, as was the case in Iceland, particularly in the 1370s (ref. 12). On the other hand, the cold made whaling a good business for European mariners who may have assaulted the Østerbygd—at least, eskimo tales blame pirates for the extinction of the weakened Norse society. Nor can it be excluded that the plague, which came to Iceland in 1402 (ref. 12), was transferred to Greenland by pirates.

Present and future climate

The dying out of the Greenland colony has fascinated generations of historians, maybe because it is the only example of a well developed European society being completely extinguished in historical time. But quantitatively it was a rather insignificant event compared to what has happened in other parts of the world—and happens today. Few of these tragedies can be ascribed to climatic changes alone, but the impact of climate on human life stands out more and more clearly, particularly where the margin of survival is slim, whether in the polar regions or in the tropics.

The warm period in the present century has culminated. In fact, most of the climatic improvement from 1920 to 1935 seems to have been lost, and although the average cooling of the Northern Hemisphere since 1940 has only been[17] of the order of 0.4 °C, it is highly significant. For example, it has reduced the growing season in England by a week or two[14] and there are indications that the small changes of the meridional temperature gradients may have induced recent disastrous events in the tropics[18].

This stresses the question of whether the future will bring back the favourable climatic conditions that prevailed in part of this century, or will remain closer to the cooler conditions that characterised most of the past six centuries or more. On the face of it, the recent warm peak was an unusual event.

The problem is, however, too important to be treated only in a speculative manner, the more so as man-made pollution of the atmosphere is now introducing new climatic parameters. Climate modelling will undoubtedly lead to a deeper understanding of the mechanism behind climatic changes and, hopefully, to the reliable climatic prognoses so badly needed for learning the consequences of man's continued impact on the environments, and for rational planning of his future activities.

In this context, the significance of palaeoclimatic records lies primarily in the need for diagnosing the processes that cause climatic changes, and for checking the validity of the models on all time scales between 10 and 10^6 yr. The record shown here offers such a possibility only for the 30–10^3 yr interval, but extension of the record 5×10^5 yr back is merely a matter of drilling deeper in Central Greenland. Nowhere else in the world is it possible to find a better combination of reasonably high accumulation rate (which ensures continuity of the record), simple ice flow pattern (which facilitates the calculation of the time scale), high ice thickness (which offers a detailed record, even at great depths) and meteorologically significant location (close to the main track of North Atlantic cyclones). This is why GISP has selected the Crête area for the drilling to the bedrock planned for 1977.

Several people contributed to the success of the GISP 1974 field season, and we are indebted to all of them: Robert Rutford and Karl Kuivinen of the GISP Management Office, University of Nebraska–Lincoln; John Rand, Lyle Hansen, Steve Mock and Chester Langway of US Army CRREL; A. Herr and P. Schmucke, Squadron VXE-6, U.S. Navy; Hans Oeschger, Bernhard Stauffer and Henry Rufli, University of Bern; Jan Nielsen and Nils Andersen, University of Copenhagen; S. E. Wølk, Danish Arctic Contractors; and Poul Madsen, Scandinavian Airlines System, Sdr. Strømfjord. We also thank W. Hibler III, H. H. Lamb and J. Namias for reading the manuscript.

This research was supported by US National Science Foundation, Office of Polar Programs; The Danish Natural Science Research Council; and the Ministry of Greenland, Copenhagen.

Received December 10, 1974; accepted February 20, 1975.

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Modified nucleosides and bizarre 5′-termini in mouse myeloma mRNA

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Messenger RNAs from mouse myeloma cells contain N^6*-methyl adenosine and novel 5′ termini having 7-methylguanosine in a 5′,5′triphosphate linkage with ribose-methylated nucleosides. Ten common 5′-terminal sequences of the forms* $m^7G^{5'}ppp^{5'}NmpNp$ *and* $m^7G^{5'}ppp^{5'}NmpNmpNp$ *are reported. Structures like this may be a general feature of mRNA in eukaryotes.*

ALTHOUGH most messenger RNAs (mRNAs) of eukaryotes are known to contain a 3′-terminal poly(A) sequence[1], no other distinctive structural features of the molecules have been identified. Almost nothing had been known about the 5′ termini, and the mRNAs were believed to contain only the four standard nucleosides, until Perry and Kelley[2] showed that mRNA from mouse L cells contains a very low level of methylated nucleosides, involving methylation of both base and ribose moieties. As it seems very likely that the rare modified nucleosides have some critical roles, we have investigated the modified residues in the poly(A)-containing mRNA from mouse myeloma cells, using a simple fingerprinting technique. We found that the pattern of modification of mRNA is very different from that of ribosomal RNA (rRNA) or transfer RNA (tRNA), that a prominent modified nucleoside in mRNA is N^6-methyladenosine, and that modified residues occur in both 5′- and 3′-terminal regions of the RNA. Our most significant finding is that the mRNAs have 5′-terminal structures of an unexpected kind, in which 7-methylguanosine and ribose-methylated nucleosides are linked by three phosphates in such a way that the terminal 7-methyl guanosine is 'inverted' with respect to the rest of the molecule. We report here ten sequences of this kind representing the 5′ termini of a large fraction of myeloma mRNAs. Related 5′-terminal structures, first found in three small nuclear RNAs of unknown function[3,4], have now also been identified in animal viral mRNAs (refs 5–8), and evidence suggests they exist in other cellular mRNAs (ref. 9). Structures of this new form may prove to be a general feature of mRNA in higher organisms.

Detection of modified nucleotides

P1 (ref. 10) or MPC 11 (ref. 11) myeloma cells were labelled in culture with ^{32}P-orthophosphate and total RNA was extracted from postnuclear (experiment 1) or postmicrosomal (experiments 2–4) supernatants. The mRNA was separated from rRNA and tRNA by two cycles of adsorption to oligo(dT)-cellulose[12] and then glycerol gradient centrifugation (see legend to Fig. 1). The RNAs were then digested with a mixture of ribonucleases[13] which produces nucleoside 3′-monophosphates (Np) from internal positions and nucleoside di-, tri-, or tetraphosphates (pNp, ppNp, or pppNp) from RNAs with 5′-terminal phosphates. The digests of RNAs containing 2′-ribose methylations will also include dinucleotides (NmpNp) or oligonucleotides ($\mathrm{Nmp(Nmp)_nNp}$), since the phosphodiester bond adjacent to a 2′-O-methyl group is resistant to the ribonucleases used. The nucleotides were fractionated by a simple modification of the Sanger fingerprinting procedure[14], involving electrophoresis on cellulose acetate at *p*H 3.5 and then on DEAE paper at the same *p*H (ref. 15).

A typical fingerprint of mouse myeloma mRNA is shown in Fig. 1*a* and is illustrated diagrammatically in Fig. 1*b*. In addition to the four major mononucleotides, a number of minor components can be seen, including a mononucleotide (designated *m1*), two prominent dinucleotides (*d1* and *d2*) and, most striking, a group of spots (*o1*–*o12*) near the top of the fingerprint in positions expected for tetra- or pentanucleotides. The fingerprint is clearly different from those of 28S and 18S rRNAs, which are characterised by high levels of many dinucleotides (Fig. 2*a* and *b*) and from that of myeloma tRNA, which contains a great variety of modified mononucleotides (Fig. 2*c*). No oligonucleotides are produced from 18S rRNA (Fig. 2*b*) or tRNA (Fig. 2*c*) and only two from 28S rRNA ('tri' and 'tetra' in Fig. 2*a*).

Bizarre 5′-terminal sequences

From the position of *o1*–*o12* on the fingerprint, the oligonucleotides were expected to contain a cluster of at least three 2′-O-methyl nucleosides (for example, NmpNmpNmpNmpNp) or to contain one or more 5′ phosphates with correspondingly fewer 2′-O-methyl nucleosides (for example, ppNmpNmpNp)—the former could be derived either from 5′ ends of the molecules or internal positions, the latter only from 5′ termini. The first type of structure was excluded when digests of the oligonucleotides were found to contain too few 2′-O-methyl nucleotides (see below). The second type was excluded when we found that alkaline phosphatase released only the 3′-terminal phosphate from each oligonucleotide and that each contained four (or less frequently, five) 'internal' phosphates. Thus we were forced to consider some much more unusual structure. One possibility was suggested by the report[3,4] that three small nuclear RNAs have 5′-terminal structures involving a previously unknown 5′,5′ diphosphate linkage,

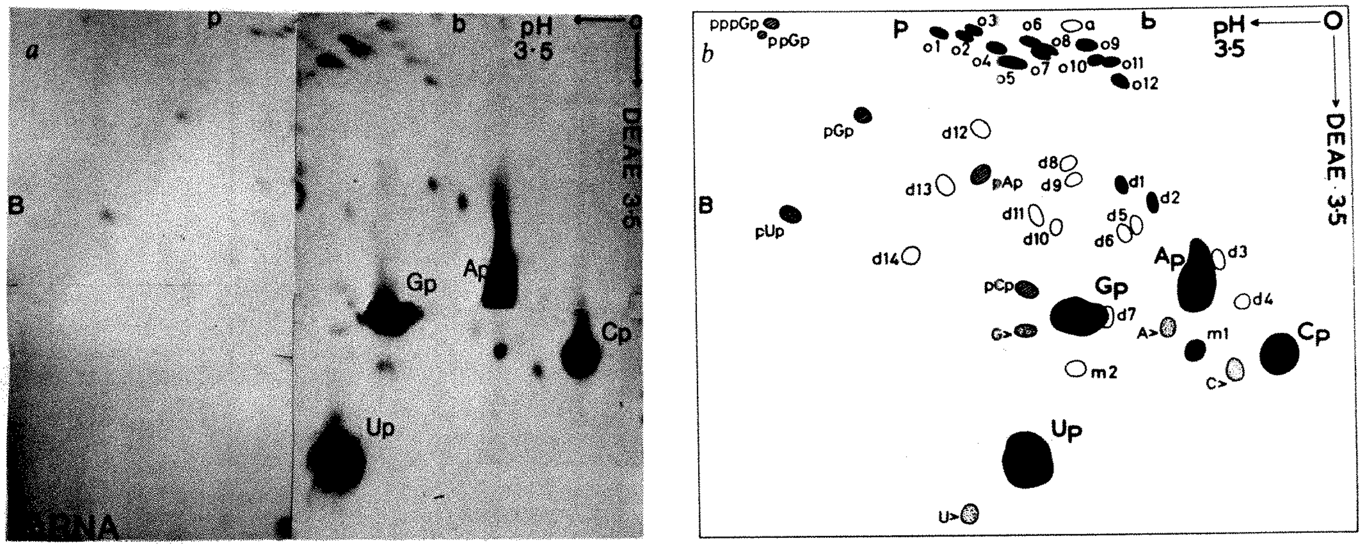

Fig. 1 Two-dimensional electropherogram of complete enzymic digest of myeloma mRNA: (*a*) 17 h autoradiograph and (*b*) schematic diagram. Poly(A)-containing RNA (35×10^6 c.p.m., 60 μg, including carrier tRNA) from a postmicrosomal supernatant of MPC 11–66.2 cells was digested for 6 h at 37 °C in 30 μl 15 mM ammonium acetate, *p*H 4.5, 2 mM EDTA with 7 μg pancreatic RNase (Worthington), 7 μg T1 RNase (Sankyo) and 20 units T2 RNase (Sankyo). The digest was vacuum dried, dissolved in 6 μl water, and subjected to high voltage electrophoresis (5 kV, Gilson Electrophorator) at *p*H 3.5 on a strip (3×90 cm) of cellulose acetate (Oxoid) in 7 M urea, 5% acetic acid, 0.5% pyridine and 2 mM EDTA until the orange G marker dye had migrated 60 cm (about 4 h)[14]. The region of cellulose acetate from the origin to just beyond the orange G marker was used for the second dimension. The nucleotides were blotted[14] on to two sheets of DEAE paper (one 46×90 cm, the other 23×90 cm), and after the sheets were washed with 70% ethanol and dried, were subjected to electrophoresis at 1.1 kV in 5% acetic acid and 0.5% pyridine (*p*H 3.5) until the xylene cyanol FF (blue) marker dye had migrated ~20 cm (about 15 h). The stippled spots in (*b*) correspond to nucleoside 2′-, 3′-cyclic phosphates. The spot marked (a) is an artefact caused by a trace of Gp sticking at the origin on the DEAE paper. *m2* is presumably also an artefact since it was absent from other fingerprints of mRNA. The letters p and b indicate the mobility of acid fuchsin (pink) and xylene cyanol FF marker dyes, respectively, in the first dimension, and B the mobility of xylene cyanol in the second dimension. Cells were labelled and poly(A)-containing RNA was isolated as follows: 1,500 ml roller cultures of MPC 11–66.2 (ref. 11) or P1Bu1 (ref. 10) cells were grown at 37 °C in Dulbecco's modified Eagle's (DME) medium (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Biocult) to 4.5×10^5 ml^{-1}. The cells were then harvested by centrifugation at 2,000 r.p.m. for 5 min (Sorvall GS3 rotor), resuspended at $5–7\times10^5$ cells ml^{-1} in phosphate-free DME medium (Gibco) supplemented with 10% heated horse serum (Biocult), and incubated at 37 °C for 3 h before addition of 50 mCi of carrier-free ^{32}Pi. After 16.5 h of labelling, which gave 30% incorporation of ^{32}Pi into TCA-precipitable material, the cultures were poured on to crushed frozen isotonic buffer, the cells harvested by centrifugation, and RNA isolated by one of two procedures. (a) Cells were lysed at 0 °C by resuspension at 10^7 ml^{-1} in 0.3% NP40, 0.13 M NaCl, 0.02 M Tris-Cl (*p*H 7.5), 7.5 mM magnesium acetate, 2 mM DTT and 0.15 M sucrose[16], and nuclei were removed by centrifugation at 0 °C for 5 min at 3,000 r.p.m. (Sorvall SS34 rotor). After a further centrifugation of the lysate at 12,500 r.p.m. for 10 min, the supernatant was adjusted to 15 mM EDTA and 0.2% SDS and digested at 0 °C for 10 min with 0.3 mg ml^{-1} proteinase K (Merck, chromatographically pure)[17]. The digest was then made 0.1 M in Tris-Cl, (*p*H 9.0) and 1% in SDS, and extracted three times at room temperature with an equal volume of phenol–chloroform–isoamyl alcohol (50:50:1, vvv)[18]. RNA was precipitated from the aqueous phase by the addition of 2.5 volumes of ethanol and then precipitated twice more from 0.2 M NaCl, 0.02 M Tris-Cl (*p*H 7.5) and 2 mM EDTA. (*b*) Cells were resuspended at 20×10^6 ml^{-1} in ice-cold hypotonic buffer (0.01 M KCl, 0.01 M Tris-Cl (*p*H 7.5) and 1.5 mM MgCl$_2$) and, after 10 min at 0 °C, were broken by nine strokes with a B pestle in a Dounce homogeniser. Ionic conditions were adjusted by the addition of 0.4 volume 2.0 M sucrose containing 62.5 mM KCl, 0.15 M Tris-Cl (*p*H 7.5) and 13.75 mM MgCl$_2$ and nuclei were removed by centrifugation as above. Microsomes were removed by sedimentation at 30,000 r.p.m. (Beckmann Type 30 rotor) for 20 min through a cushion of 5.75 ml 0.8 M sucrose in 0.1 M KCl, 0.05 M Tris-Cl (*p*H 7.5) and 5 mM MgCl$_2$ (ref. 19). The entire post-microsomal supernatant was diluted twofold with distilled water, then digested with proteinase K and extracted as in (*a*). Poly(A)-containing RNA was separated from bulk RNA by two cycles of oligo(dT) chromatography at 30 °C. RNA was applied at 3–4 A_{260} ml^{-1} in 0.3 M NaCl, 0.1% SDS, 0.01 M Tris-Cl(*p*H 7.5) and 2 mM EDTA to a (1×2 cm) column of oligo(dT)–cellulose[12]. The column was then washed thoroughly with starting buffer followed by 0.2 M NaCl, 0.01 M Tris-Cl (*p*H 7.5) and 2 mM EDTA. The poly(A)-containing RNA (~ 1×10^6 c.p.m. μg^{-1}), representing about 2% of the A_{260}, was then eluted with 0.01 M Tris-Cl (*p*H 7.5) and 2 mM EDTA, rechromatographed as above, and finally precipitated with ethanol in the presence of carrier tRNA. The RNA was dissolved in KTE buffer (0.01 M KCl, 0.01 M triethanolamine-Cl (*p*H 7.5) and 1 mM EDTA, heated to 65 °C for 5 min[19], chilled rapidly and then sedimented at 40,000 r.p.m. (Beckmann SW 40 rotor) for 12.5 h through a 10–30% glycerol gradient in KTE buffer. Small amounts of material sedimenting at the same positions as marker 28S and 18S rRNAs and very small amounts less than 9S in size were discarded, and the remaining RNA was recovered by ethanol precipitation and reprecipitated twice more before digestion.

for example, m$_3$G$^{5'}$pp$^{5'}$AmpUmpC in the U2 RNA, where m$_3$G is N^2,N^2,7-trimethylguanosine[4,21].

We have now established that the oligonucleotides in mRNA have structures of the forms m^{7}G$^{5'}$pp$^{5'}$NmpNp and m^{7}G$^{5'}$ppp$^{5'}$NmpNmpNp, that is, 7-methylguanosine linked through its 5′ carbon by three phosphates to the 5′ carbon of a 2′-O-methylnucleoside, followed in some sequences by a second 2′-O-methylnucleoside, and then by an unmodified nucleoside (Fig. 3). Note that since the linkage of 7-methylguanosine and the first 2′-O-methylnucleoside is 5′, 5′ rather than the 3′, 5′ of conventional phosphodiester bonds, the 7-methylguanosine has a free 3′ hydroxyl.

The evidence for these structures, which can occur only at the 5′ termini of the mRNAs, will be presented elsewhere but in part is as follows: (1) Limited digestion of each oligonucleotide with venom phosphodiesterase, which only readily attacks oligonucleotides having a 3′-hydroxyl[22], released 7-methylguanosine 5′-phosphate, that is by the scission m^{7}G$^{5'}$p/pp$^{5'}$Nmp Np or m^{7}G$^{5'}$p/pp$^{5'}$NmpNmpNp. This indicates that the m^{7}G has a free 3′ hydroxyl and is linked through its 5′ phosphate. Moreover, this cleavage exposed two 5′ phosphates on the other product (pp$^{5'}$NmpNp or pp$^{5'}$NmpNmpNp), since alkaline phosphatase now released 3 mol Pi from it. Thus the m^{7}G is joined to the rest of the oligonucleotide by a 5′, 5′ triphosphate linkage. (2) Nucleotide pyrophosphatase, which liberates N^2, N^2, 7-trimethylguanosine 5′ phosphate from the related structures in the small nuclear RNAs[3,4], cleaves 7-methylguanosine 5′phosphate from the oligonucleotides, giving the same products as above. (3) Complete digestion of each oligonucleotide with venom phosphodiesterase after removal of the 3′ phosphate gives the expected products: pm^{7}G from the 5′ end, a pN from the 3′ end, either one or two pNm from internal positions and

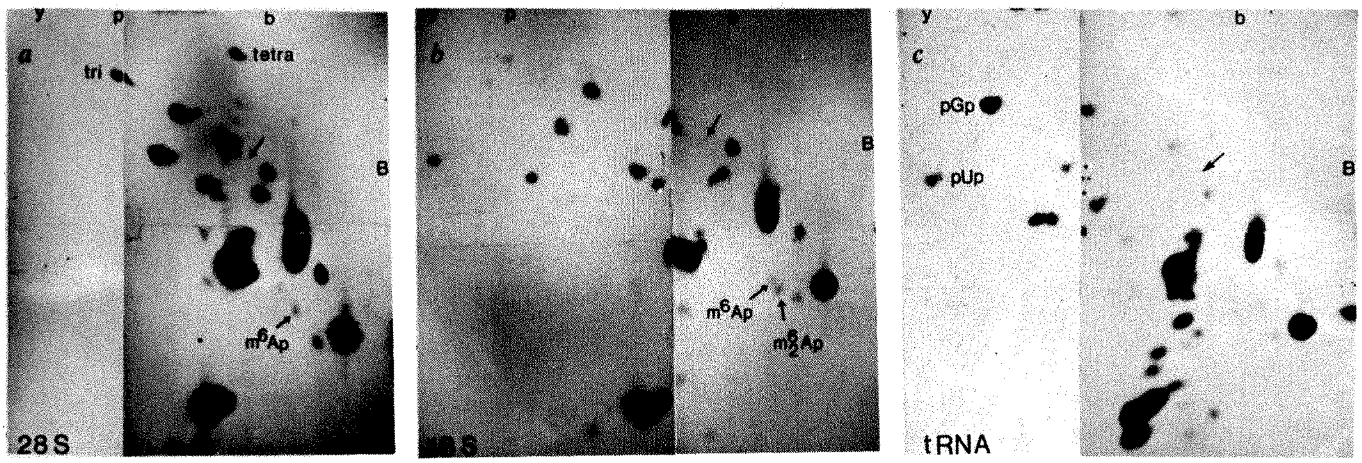

Fig. 2 Two-dimensional electropherograms of complete enzymic digests of (*a*) 28S rRNA (60 μg); (*b*) 18S rRNA (60 μg); (*c*) tRNA (30 μg) from MPC 11-66.2 myeloma cells. Digestion and electrophoresis conditions were as described in Fig. 1 and autoradiography was for 17 h. 28S, 18S and 3-8S RNA fractions were prepared by glycerol gradient centrifugation of the RNA which did not absorb to oligo(dT)-cellulose (see legend to Fig. 1). The 28S and 18S rRNA fractions (specific activity ~ 2.7×10^5 c.p.m. μg^{-1}) were purified further by a second gradient centrifugation. The tRNA was purified by subjecting the 3-8S RNA fraction to electrophoresis on a 10% polyacrylamide slab gel and extracting the entire region of the gel containing molecules 70–90 nucleotides in length[20]. Y indicates the mobility of the orange G dye marker in the first dimension and those of the other dyes are indicated as in Fig. 1.

Pi (for example, $m^7G^{5'}p/p/p^{5'}Nm/pN$). The digests also frequently contained some ppNm, which is compatible with a triphosphate but not a diphosphate linkage. For instance, digests of dephosphorylated *o5* contained pm^7G, pU, pCm, Pi and some ppCm, so we assign it the structure $m^7G^{5'}ppp^{5'}CmpU$.

Rottmann *et al.*[9] have recently proposed related structures for oligonucleotides from L cell mRNA: *XppNmpNp and *XppNmpNmpNp, where *X is an unidentified blocking group, possibly a nucleoside of 7-methlyguanosine type in 5′,5′diphosphate linkage with either one or two 2′-O-methyl nucleosides.

We have determined the sequences of the ten most common 5′-terminal oligonucleotides from myeloma mRNA (Table 1) and we have tentative sequences for ten others (J.M.A. and S.C., unpublished). In every sequence, the 5′-terminal nucleoside seems to be 7-methylguanosine and no derivatives of m^7G, such as the trimethylguanosine of the small nuclear RNAs[4,21], have been detected. The m^7G is in 5′, 5′ triphosphate linkage in all the sequences and none having a diphosphate linkage has been detected. All four standard 2′-O-methylnucleosides are present and a fraction of the 2′-O-methyladenosine in oligonucleotides *o6a*, *o9* and *o10* seems to contain a modified base. No evidence of other base modifications has as yet been found.

The relative molar frequency of the oligonucleotides is also given in Table 1. Clearly certain sequences are much more abundant than others; the most common sequence, $m^7G^{5'}ppp^{5'}$ CmpUp, represents about 20% of the modified termini and this and four other sequences constitute about 60% of these end groups. Sequences having one ribose methylation are much more abundant than those with two (about 6:1) but a number of the minor sequences are of the latter type (J.M.A. and S.C., unpublished). The 2′-O-methylnucleoside involved in triphosphate linkage can be any of the four standard ones, but sequences with Cm, Am, and Gm in this position are much more prevalent than those with Um. The relative frequency of 5′ termini in mRNA from microsomes seems to be similar to that in mRNA from postmicrosomal supernatants.

The proportion of mRNAs containing the oligonucleotides can be assessed from the data in Table 2, which gives their yields (and those of other modified nucleotides) expressed as mol per 10^4 residues and mol per 1,600 residues. The latter is an estimate for the number average size of the myeloma mRNAs based on the measured fraction (9.3%) of the mRNA radioactivity present in poly(A) sequences (average of four experiments) and a number average size of 150 residues for the labelled poly(A) determined by polyacrylamide gel electrophoresis in 8 M urea. The overall yield of the oligonucleotides has varied between 0.53 and 0.86 mol per 1,600 residues in four mRNA preparations, probably depending on the intactness of the mRNA. Since the yield was close to 1 mol per

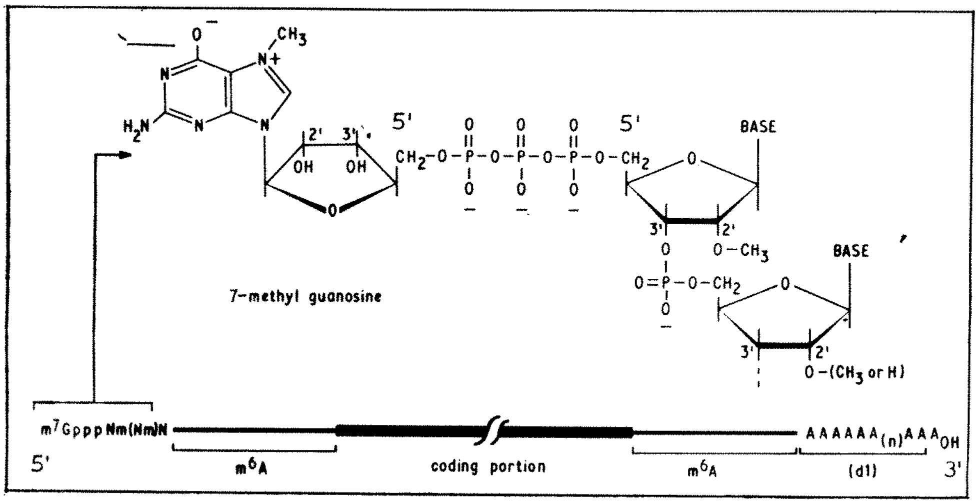

Fig. 3 5′-Terminal structure and possible location of the major modified nucleosides in myeloma mRNA. Note that the assignment of m^6A to two positions in the diagram may not mean that every molecule has two residues of m^6A. *d1* is shown in parentheses since only a fraction of the molecules contain it (see Table 2).

1,600 residues in two mRNA preparations (experiments 3 and 4 in Table 2), however, we conclude that a very large fraction of myeloma poly(A)-containing mRNAs (probably all) contain one of these modified 5′-terminal sequences. This conclusion is weakened only if the average size of the mRNA is actually much less than 1,600 residues, which seems unlikely. Most estimates for the average size of mRNAs in mammalian cells are in fact higher than this[23–26].

Since there can be no more than 1 mol of a 5′-terminal oligonucleotide per chain, the amounts of these products found in experiments 3 and 4 enable us to set an upper limit of about 1,900 residues for the number average size of the myeloma mRNAs. Setting this upper limit does not require our conclusion concerning the proportion of the mRNAs having modified 5′ termini.

Nucleoside diphosphates (pNp) have also been detected in the mRNA digests (Fig. 1*b*), but the yields have been very variable and were lowest in the two mRNA preparations in which the highest yields of oligonucleotides were found (Table 2). Therefore it seems likely that these 5′-end groups are derived primarily from mRNA which has been degraded either *in vivo* or during its isolation. A fraction of the pNp may be an artefact of the digestion (or other procedures), since we found some spurious pAp in digests of poly(A). Traces of ppGp and pppGp (Fig. 1*b*) were found in two experiments but not in two others (Table 2). No other ppNp or pppNp has been detected.

Other modified nucleotides

We have identified the prominent modified mononucleotide *m1* in Fig. 1*b* as N^6-methyladenylic acid (m^6Ap). A nucleotide in exactly the position of *m1* in fingerprints of 28S and 18S rRNA (Fig. 2*a* and *b*) was known to be m^6Ap (refs 27–29 and J.M.A. and S.C., unpublished) and *m1* had the same mobility as this nucleotide[30] on thin layer chromatography in five solvent systems. Since our procedures do not involve the alkaline conditions which produce m^6A from N^1-methyladenosine (m^1A)[31] and did not produce m^6A from m^1A in myeloma tRNA, it is clear that the mRNA contains the N^6-derivative. The average yield of m^6Ap is 1.3 mol per 1,600 residues (Table 2), so every mRNA could contain at least one residue of m^6A. No other modified mononucleotides have as yet been detected in mRNA digests, although our procedures reveal at least a dozen of the modified mononucleotides in tRNA digests (Fig. 2*c*). Since completion of this work, Desrosiers *et al.*[32] have reported that m^6A (and/or m^1A) is the only prominent nucleoside with a methylated base in Novikoff hepatoma mRNA.

Dinucleotides *d1* and *d2* (Fig. 1), isolated in submolar amounts from mRNA (Table 2), are not standard NmpNp. No product corresponding to *d1* occurs in rRNA or tRNA (arrows in Figs 2*a–c* indicate where *d1* would be). *d1* is alkali-stable and our analysis to date suggests the structure N*mpAp, where N*m is an unusual 2′-O-methylated adenosine derivative. *d2* is in the position of AmpAp but lacks a 2′-O-methyl ribose since it is hydrolysed by alkali, giving Ap and Xp, where Xp probably contains a modified adenine (not m^6A, m_2^6A, or the base present in *d1*).

Dinucleotides *d3–d14* in Fig. 1 are the standard NmpNp found in high yield in rRNA (refs 15, 27, 28, 33 and J.M.A. and S.C., unpublished). Since they were virtually absent in two mRNA preparations, *d3–d14* clearly come from rRNA. The level of NmpNp thus provides a quantitative chemical index of contamination by rRNA, which we calculate to be about 2.5% in the mRNA used in Fig. 1*a* but less than 0.5% in experiments 3 and 4 of Table 2.

If we arbitrarily assign *d1* and *d2* one methyl group each, we can calculate from the yields of all the products so far detected (Table 2) that myeloma mRNA contains about 2.0 methylated nucleosides per 1,000 residues. As this is very close to the 2.2 ^3H-methyl groups per 1,000 residues found in mouse L cell mRNA (ref. 2), it seems likely that the major methylated nucleosides in the mRNA have been accounted for.

Table 1 Common 5′-terminal sequences in myeloma mRNA

Oligonucleotide	Structure*	Relative molar frequency† of modified termini
o5	$m^7Gp^{5'}pp^{5'}CmpUp$	21%
o7	$m^7Gp^{5'}pp^{5'}GmpCp$	11%
o6a‡	$m^7Gp^{5'}pp^{5'}AmpGp$	12%
o6b‡	$m^7Gp^{5'}pp^{5'}GmpAp$	6%
o3	$m^7Gp^{5'}pp^{5'}GmpGp$	8%
o12	$m^7Gp^{5'}pp^{5'}CmpCp$	5%
o8§	$m^7Gp^{5'}pp^{5'}CmpUmpCp$	6%
o1	$m^7Gp^{5'}pp^{5'}GmpUp$	5%
o10	$m^7Gp^{5'}pp^{5'}AmpCp$	5%
o9	$m^7Gp^{5'}pp^{5'}AmpAp$	4%
	Total	83%

*The derivation of the sequences will be reported elsewhere (S. Cory, and J. M. Adams, unpublished).

†The relative molar frequency was calculated from the data in Table 2.

‡The analysis of *o6* indicated the presence of the isomers in a ratio of about two to one.

§The analysis of *o8* indicated the presence of $m^7Gp^{5'}pp^{5'}CmpUmpCp$ but did not exclude the presence of $m^7Gp^{5'}pp^{5'}UmpCmpCp$ as well.

Preliminary location experiments

We have carried out two types of experiments to map the positions of m^6A, *d1* and *d2* within the mRNA molecules. In the first, mRNA was treated with 0.1 M NaOH for 4 min at 0 °C, which gave on average about one scission per molecule; 3′-terminal poly(A)-containing 'halves' (and residual intact molecules) were then separated from 5′-terminal 'halves' by passage through oligo(dT)-cellulose. In the second, mRNA was digested to completion with T1 RNase (which splits after Gp residues) and oligo(dT)-cellulose was used to separate the poly(A) fragments, which include a few residues before the poly(A) sequence[34,35], from oligonucleotides derived from the rest of the molecule. Each of the four sets of fragments was then fingerprinted as described previously.

The results obtained were consistent with the model shown at the bottom of Fig. 3. As expected, all the oligonucleotides were enriched in the 5′-terminal 'halves' and completely absent from the 3′-terminal T1-poly(A) fragment. *d1* was present only in the two sets of fragments containing poly(A) and moreover, was present in a fragment from which all residues preceding the poly(A) sequence had been excised with pancreatic RNase. Thus *d1* must be contiguous with or within the poly(A) sequence. Neither m^6A nor *d2* was present in the T1-poly(A) fragments, but m^6A (and apparently *d2*) was present in similar amounts in both 5′- and 3′- 'halves'. Conceivably, m^6A may be located within the coding portion of the molecule, but as its hydrogen bonding properties[36,37] may interfere somewhat with translation, we favour a location within the non-coding regions of mRNA, which are thought to include a 5′-terminal region and a region preceding the poly(A) sequence[1,38].

Significance

In summary, poly(A)-containing mRNA from mouse myeloma cells contains at least eight kinds of modified nucleosides, including 7-methylguanosine, N^6-methyladenosine, the four standard ribose-methylated nucleosides and smaller

Table 2 Yields of minor nucleotides from mouse myeloma mRNA

Product (no.of phosphate residues)	Mol per 10^4 residues* Expt 1†	Expt 2‡	Expt 3‡	Expt 4‡	Average mol per 1,600 residues
o1 (5)	0.22	0.18	0.23	0.33	0.04
o2 (6)	0.13	0.10	0.13	0.26	0.02
o3 (5)	0.38	0.29	0.38	0.45	0.06
o4 (5)	0.18	0.11	0.21	0.18	0.03
o5 (5)	0.85	0.94	1.10	0.80	0.15
o6 (5)	0.70	0.57	1.00	0.94	0.13
o7 (5)	0.44	0.27	0.69	0.62	0.08
o8 (6)	0.22	0.20	0.24	0.36	0.04
o9 (5)	0.14	0.11	0.27	0.22	0.03
o10 (5)	0.19	0.13	0.30	0.27	0.04
o11 (6)	0.05	0.07	0.03	0.08	0.01
o12 (5)	0.18	0.24	0.29	0.25	0.04
minor (6)	~0.29	~0.11	~0.53	~0.43	~0.05
Total	3.97	3.32	5.40	5.19	0.72
pGp	0.51(0.93)§	0.20	ND	ND	—
pAp	0.11(0.88)§	0.86	0.52	1.17	—
pUp	0.38(1.1)§	0.79	0.23	ND	—
pCp	ND	0.66	0.23	ND	—
ppGp	0.08	ND	ND	ND	—
pppGp	0.21	0.10	ND	ND	—
Total	1.29(3.20)	2.61	0.98	1.17	—
di (2)	1.4	1.0	0.63	1.6	0.19
d2 (2)	0.85	1.6	1.4	0.67	0.18
m1 (1)	9.0	8.2	6.1	8.6	1.3

*Molar yields were determined by measuring the radioactivity in the paper containing each nucleotide in a scintillation counter, dividing by the total radioactivity in all the products, and correcting for the number of phosphates per nucleotide shown in parentheses in the first column. In practice, to compensate for some variability in the transfer of Cp to DEAE paper, we related the yield of each minor nucleotide to Up and multiplied by a factor determined from the known frequency of Up (23.35%), measured by an independent method.

†Experiment 1 is a digest of poly(A)-containing RNA from a post nuclear supernatant of P1Bu1 myeloma cells prepared by procedure (*a*), (see legend to Fig. 1). The yields were measured for two separate digests of the same RNA preparation and have been averaged except where they differed by more than 25%.

‡Experiments 2, 3 and 4 are digests of poly(A)-containing RNA from post microsomal supernatants of MPC 11-66.2 myeloma cells, prepared by procedure (*b*) (experiment 2; see legend to Fig. 1), or by a modification of the double detergent procedure[16] (experiments 3 and 4). The fingerprint for experiment 2 is shown in Fig. 1*a*.

§The much higher yield of pNp in the second fingerprint of the same mRNA preparation is probably caused by some artefactual production of new 5′-terminal phosphates (see text).

ND, not detected.

amounts of two unidentified components (in *d1* and *d2*). A myeloma mRNA of 1,600 nucleotides contains three to four modified residues, possibly arranged as shown at the bottom of Fig. 3. The 7-methylguanosine and the standard 2′-O-methylnucleosides are found only in 5′-terminal structures, where they are joined in an unexpected 5′, 5′ triphosphate linkage. Since the 7-methylguanosine is thus 'inverted' with respect to the remainder of the chain, the mRNAs, surprisingly, have free 3′- and 2′-hydroxyls at each end of the molecule. At least 20 and possibly 30 different modified 5′-terminal sequences are present in myeloma mRNA, but all seem to be of the two forms m^{7}G$^{5'}$ppp$^{5'}$NmpNp and m^{7}G$^{5'}$ppp$^{5'}$NmpNmpNp, with the former occurring much more frequently. Ten sequences accounting for about 80% of the 5′ termini have been determined and certain sequences are found to greatly predominate, with five of the known sequences representing about 60% of the total.

The various modified 5′ termini in mRNA may assume different physical structures. Base stacking and other interactions between the 7-methylguanosine and the different 2′-O-methyl nucleosides would be facilitated by the additional flexibility of a 5′, 5′-triphosphate bridge[39]. Presumably the unusual charge properties of 7-methylguanosine[40] influence the structures.

The modified 5′-termini provide the first chemical marker for this region of mRNA. Moreover, the presence of 2′ and 3′ hydroxyls on the terminal nucleoside permits labelling of 5′ termini with ^{3}H-borohydride[4,5] and should allow other reactions normally restricted to 3′ termini of RNA molecules[41].

5′-Terminal structures identical in form to those described here have very recently been identified in animal viral mRNAs synthesised *in vitro*. Furuichi *et al.*[5] report that all the mRNAs made by reovirus particles in the presence of the methyl donor S-adenosyl-methionine contain the sequence m^{7}G$^{5'}$ppp$^{5'}$GmpCp and mRNAs synthesised similarly by particles of the insect cytoplasmic polyhedrosis virus (CPV), which also has a double-stranded RNA genome, have m^{7}Gp$^{5'}$pp$^{5'}$AmpGp[6]. Moreover, Wei and Moss[7] have shown that the mRNAs made by particles of vaccinia virus, which has a DNA genome, have the 5′-terminal sequences, m^{7}G$^{5'}$ppp$^{5'}$GmpNp and m^{7}G$^{5'}$ppp$^{5'}$AmpNp, although the same sequences with a diphosphate linkage have been suggested by others[8].

We suspect that 5′-terminal structures of this new form will prove to be a general feature of mRNA in many, if not all, eukaryotic cells and for a variety of viral mRNAs. It seems probable that these complex structures are required for some general process(es) concerning mRNA, such as processing from precursors, transport to the cytoplasm or translation. The viruses cited above replicate in the cytoplasm, so we infer that at least one function of the structures is in a cytoplasmic process. Since there is very high sequence specificity in the methylation of RNA (refs 42 and 43), a considerable number of methylating enzymes presumably is required to generate the variety of methylated 5′-terminal sequences found in the cellular mRNA; this suggests that the particular modified sequences at the 5′ termini are important in their function. They may, for example, serve to 'tag' mRNAs (or mRNA precursors) to be handled in different ways.

The terminal 5′, 5′ linkages in mRNAs cannot, of course, be formed by normal transcriptional events; they must instead form by a special 5′, 5′ condensation reaction between a 5′-phosphorylated guanosine derivative and another nucleotide or polynucleotide, probably also 5′-phosphorylated. For some viral RNAs this reaction occurs during transcription. The modified 5′ termini of CPV mRNA (ref. 44) and probably also reovirus mRNA (refs 5 and 45) are formed on very small nascent chains by a reaction in which the 7-methylguanosine and adjacent phosphate originate from GTP and the other two phosphates from the NTP initiating chain formation[6]. Both ribose and base methylations seem to be closely coupled to the condensation reaction[5].

The reactions involved in the formation of the modified 5′ termini of cellular mRNAs are not known, nor is it even established whether they occur in the nucleus. Seemingly the reactions must differ from those for the viral mRNAs cited above, which are formed in the cytoplasm as primary transcription products. The mRNAs of mammalian cells are thought to be derived from 3′-terminal portions of longer precursors among the heterogeneous nuclear RNAs (ref. 1); the cleavage of the precursor would thus produce a mRNA segment lacking the 5′-terminal pppN . . . sequences of primary transcripts[46]. Conceivably the ribose methylation of mRNA precursors marks the site for this cleavage and the 5′, 5′ condensation reaction occurs either during or subsequent to the cleavage (see also refs 5 and 9). Since the location of mRNA sequences near the 3′ termini of nuclear precursors is not firmly established, however, the possibility that the 5′-terminal sequences in the mRNAs correspond to the 5′ termini of primary transcripts, as in the viral mRNAs, cannot be completely excluded.

We thank Drs T. S. Ro-Choi, A. J. Shatkin and C. M. Wei for providing manuscripts; Drs R. Cotton and M. D. Scharff for strains; Dr A. W. Harris for help with culture conditions; Drs L. R. Finch, K. D. Shortman and A. W. Harris for comments on the manuscript; and R. Muller and H. Simpson for assistance. This work was supported by grants from the

National Cancer Institute, the American Heart Association, the Anna Fuller Foundation, the NH and MRC (Canberra) and the ARGC. J.M.A. is an Established Investigator of the American Heart Association and S.C. a Roche Fellow.

Received October 18, accepted March 27, 1975.

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5′-Terminal 7-methylguanosine in eukaryotic mRNA is required for translation

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Unmethylated reovirus and VSV mRNAs are specifically methylated to form 5′-terminal structures of the type, m^7G (5′) ppp (5′) N by protein synthesising extracts prepared from wheat germ and mouse L cells. Reticulocyte mRNA also contains 5′-terminal m^7G. mRNAs having 5′-terminal m^7G stimulate protein synthesis in vitro. *Removal of m^7G by β-elimination abolishes translation of the mRNAs.*

REOVIRUS and vesicular stomatitis virus (VSV) mRNAs synthesised *in vitro* by their virion-associated RNA polymerase in the presence of S-adenosyl-methionine (SAM) contain the 5′-terminal structures, $m^7G(5')ppp(5')G^m$ (ref. 1) and m^7G (5′) ppp (5′) A^m (ref. 2), respectively, and no other methylated nucleotides. The mRNAs are translated with fidelity in a wheat germ cell-free protein synthesising system[3,4], and their messenger activity is unaffected by addition to the extract of SAM or its analogue, S-adenosyl-homocysteine (SAH)[5]. Viral mRNA made in the absence of SAM also promotes the synthesis of authentic viral polypeptides *in vitro*; this was shown to be due to the ability of the wheat germ extract to methylate the viral mRNAs since translation of unmethylated viral mRNA was enhanced by addition of SAM to the extract but completely blocked by the inhibitor of methylation, SAH[5]. To determine if the methylation of mRNA required for translation is specific, we have analysed reovirus and VSV mRNAs after incubation in cell-free protein synthesising extracts. The viral mRNAs are methylated exclusively at the 5′ termini and the resulting 7-methyl-guanosine (m^7G) is essential for translation. Similarly, rabbit reticulocyte mRNA contains 5′-terminal m^7G and its removal by β-elimination results in the loss of translational activity *in vitro*.

5′-Terminal methylation of mRNA

Reovirus mRNA was synthesised with α-^{32}P-CTP as radioactive precursor in the presence of SAH to minimise methylation during transcription[6]. The 5′ termini of reovirus mRNA synthesised in these conditions are predominantly ppG . . ., but some of the molecules (~ 25%) have blocked, unmethylated 5′-terminal GpppG (our unpublished results). Purified, ^{32}P-labelled mRNA was incubated in a wheat germ cell-free extract with methyl-^3H-SAM in conditions of protein synthesis as described previously[3–5]. The mRNA was extracted with phenol, separated from 4S RNA by density gradient centrifugation[5], digested with *Penicillium* nuclease and alkaline phosphatase and analysed by high voltage paper electrophoresis[1]. All the ^3H derived from methyl-^3H-SAM by methylation of the RNA migrated as a single component in the region characteristic of blocked 5′-terminal structures[1,7] (Fig. 1*a*). No ^3H-labelled nucleosides were detected, consistent with the absence of methylated residues within the RNA chains. The enzymatic digestions were complete as shown by the quantitative recovery of the ^{32}P as Pi. The presumptive 5′-terminal material was analysed by DEAE-cellulose column chromatography and had a net negative charge of −2.5 (Fig. 1*b*, inset). The same charge was found for m^7GpppG^m isolated from the 5′ termini of methylated reovirus mRNA. When analysed by paper chromatography, however, the presumptive 5′ termini (peak I) migrated more slowly than marker m^7GpppG^m (Fig. 1*b*). Treatment of the peak I material with nucleotide pyrophosphatase converted all the radioactivity to 7-methylguanylic acid (m^7pG); no 2′-O-methylguanylic acid (pG^m) or other methylated nucleotides were detected (Fig. 1*c*). Alkaline phosphatase treatment after pyrophosphatase digestion converted the radioactivity to m^7G (Fig. 1*d*). The identity of both m^7pG and m^7G was confirmed by paper chromatography as described previously[1]. The results indicate that in the presence of SAM, wheat germ extract methylates the 5′ termini of reovirus mRNA, yielding m^7GpppG. This blocked, methylated structure is similar to the 5′-terminal sequence of reovirus mRNA methylated during transcription[1], but with the notable absence of 2′-O-methylation.

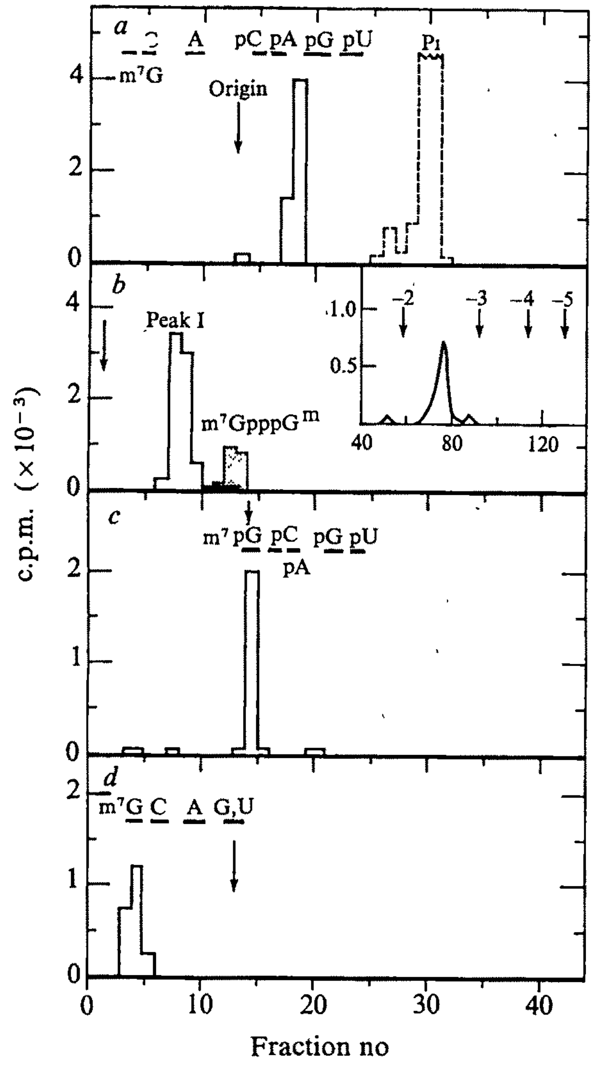

Fig. 1 Methylation of 5′ termini of reovirus mRNA by wheat germ extract. Reovirus mRNA (20 μg) synthesised in the presence of α-^{32}P-CTP and 10 μM SAH was incubated for 20 min in wheat germ cell-free protein synthesising extract containing methyl-^{3}H-SAM (2 μM, 7.3 Ci mmol^{-1})[3,5,6]. ^{32}P-labelled viral mRNA was recovered by phenol extraction and glycerol gradient centrifugation[3]. *a*, After treatment of the RNA with *Penicillium* nuclease followed by alkaline phosphatase, the digest was analysed by high voltage paper electrophoresis (pyridine acetate, *p*H 3.5, 2600 V, 40 min)[1]. *b*, The enzyme-resistant ^{3}H-labelled material in *a* was eluted and analysed by descending paper chromatography in isobutyric acid: 0.5N NH_4OH (10:6 v/v). Inset: an aliquot of peak I material was mixed with 20 A_{260} of a pancreatic RNase digest of yeast tRNA and applied to a 1×20 cm column of DEAE-cellulose equilibrated with 0.05 M Tris buffer, *p*H 8, containing 7 M urea. The column was eluted with a gradient of NaCl (0.0–0.3 M). Samples of 1 ml were counted in methyl-cellosolve and toluene-based scintillant[7]. The arrows represent the positions of the oligonucleotide markers as determined by absorbancy. *c*, Peak I material was digested with nucleotide pyrophosphatase and analysed by paper electrophoresis[1]. *d*, Peak I material was treated with a mixture of nucleotide pyrophosphatase and alkaline phosphatase and analysed by electrophoresis. The papers were dried, cut into 1 cm strips, and counted in toluene-based scintillation fluid[1].

To determine if VSV mRNA is also methylated at the 5′ terminus by wheat germ extract, ^{32}P-labelled viral mRNA was synthesised *in vitro* in the absence of SAM; in these conditions the 5′ termini of the transcription products are GpppA (ref. 8). The RNA was purified and incubated in the wheat germ cell-free system with methyl-^{3}H-SAM. After *Penicillium* nuclease plus alkaline phosphatase digestion of the repurified VSV mRNA, a single ^{3}H-labelled component which migrated toward the anode was obtained by paper electrophoresis (Fig. 2*a*). Paper chromatography of this presumptive 5′-terminal material again revealed that it migrated more slowly than marker m^{7}GpppA^m, the 5′-terminal structure of VSV mRNA methylated by the virion-associated enzyme[2] (Fig. 2*b*). Digestion with nucleotide pyrophosphatase and alkaline phosphatase converted all the ^{3}H-labelled material to m^{7}G and, as in reovirus mRNA, no 2′-O-methylated nucleosides were obtained (Fig. 2*c*). The structure of the m^{7}G was confirmed by paper chromatography. Thus, in the presence of SAM, reovirus and VSV mRNAs are methylated exclusively at the 5′ ends by wheat germ extract, yielding the 5′-terminal structures, m^{7}GpppG and m^{7}GpppA, respectively.

The translation of animal virus mRNAs in wheat germ extracts represents a heterologous system, and it was interesting to determine whether cell-free extracts from uninfected animal cells also contain viral mRNA methylating activity. Mouse L cells were used since they replicate reovirus, and cell-free extracts prepared from them have been shown to translate reovirus mRNA[9,10] and VSV mRNA (G. W. B., A. K. Banerjee, and A. J. S., unpublished results). An L cell protein synthesising S10 extract[10] was incubated with ^{32}P-labelled, unmethylated reovirus mRNA and methyl-^{3}H-SAM. The mRNA was methylated as shown by glycerol density gradient centrifugation, and the kinetics of methylation were similar to those observed with the wheat germ extract[5] (data not shown). The *Penicillium* nuclease plus alkaline phosphatase digestion products of the methylated mRNA isolated from the L cell extract were analysed by paper electrophoresis (Fig. 3*a*). The ^{3}H-labelled, presumptive 5′-terminal material migrated between pA and pG,

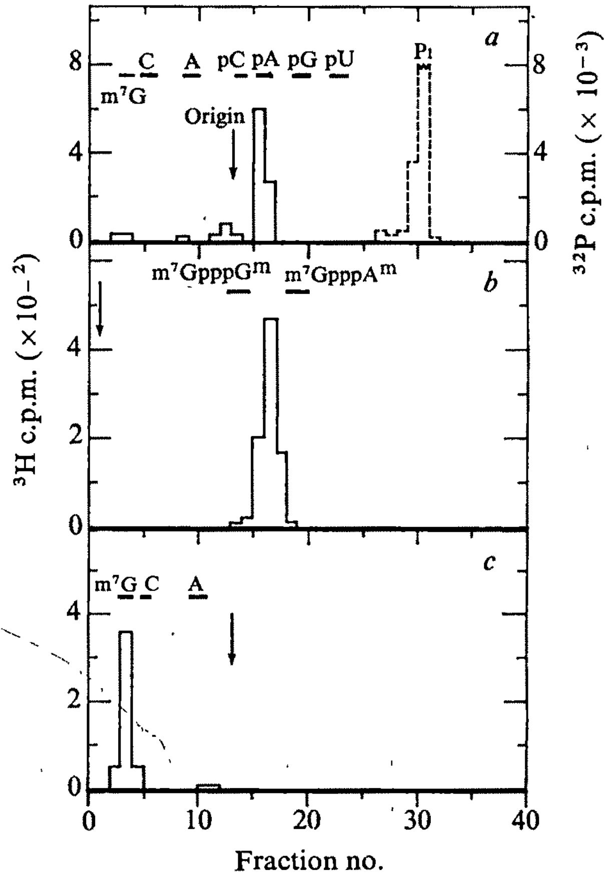

Fig. 2 Methylation of VSV mRNA 5′ termini by wheat germ extract. VSV mRNA (5 μg) synthesised *in vitro*[20] in the presence of α-^{32}P-GTP was incubated with methyl-^{3}H-SAM and analysed as in Fig. 1. *a*, Paper electrophoresis of *Penicillium* nuclease plus phosphatase digest; *b*, paper chromatography of enzyme-resistant, ^{3}H-labelled material from *a*; *c*, paper electrophoresis of nucleotide pyrophosphatase plus phosphatase digest of material in *b*. Marker compounds m^{7}GpppG^m and m^{7}GpppA^m were purified from *Penicillium* nuclease plus alkaline phosphatase digests of methylated mRNAs of reovirus[1] and cytoplasmic polyhedrosis virus[13], respectively.

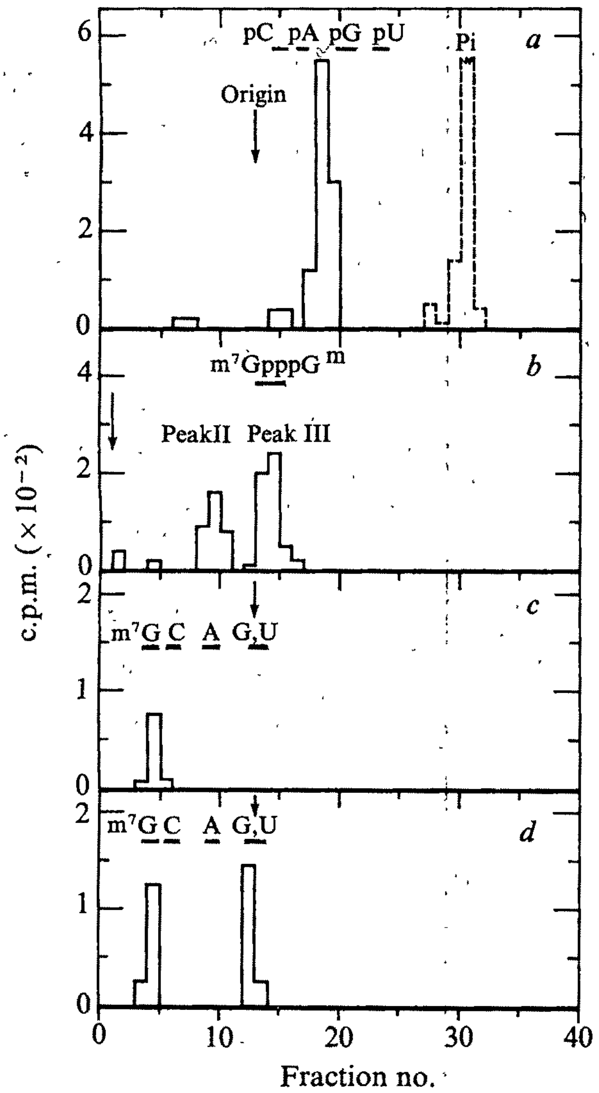

Fig. 3 Methylation of reovirus mRNA 5′ termini by L cell extract. Reovirus mRNA (10 μg) made in the presence of α-^{32}P-CTP and 10 μM SAH[3,6] was incubated under conditions of protein synthesis for 20 min in L cell extract[10] with methyl-^{3}H-SAM (3.4 μM, 7.3 Ci mmol^{-1}). The RNA was isolated and analysed as in Fig. 1. *a*, Paper electrophoresis of *Penicillium* nuclease and alkaline phosphatase digest; *b*, paper chromatography of enzyme-resistant, ^{3}H-labelled material in *a*; *c*, electrophoresis of peak II material after digestion with nucleotide pyrophosphatase and phosphatase; *d*, peak III material analysed as in *c*.

but by paper chromatography two components could be resolved (Fig. 3*b*). The faster component (peak III) migrated with m^{7}GpppG^{m} and the second component (peak II) migrated more slowly, as observed for m^{7}GpppG (peak I, Fig. 1*b*). Incubation with nucleotide pyrophosphatase plus alkaline phosphatase converted all the radioactivity in peak II to m^{7}G (Fig. 3*c*). By contrast, enzyme treatment of peak III yielded ^{3}H-labelled m^{7}G and an equal amount of radioactivity migrating in the position of G (Fig. 3*d*), which was identified as 2′-O-mG by paper chromatography. Therefore, cell-free extracts of both wheat germ and L cells contain enzyme activities that specifically methylate the N^{7} position of the 5′-terminal guanosine. L cell extracts, however, can also methylate the 2′-OH of the 5′ penultimate guanosine in reovirus mRNAs.

Dependence of translation on 5′-m^{7}G

Translation of reovirus and VSV mRNA by wheat germ extracts has been shown to depend on methylation of the mRNA (ref. 5). From the results shown in Figs 1 and 2, it is likely that N^{7} methylation of the 5′-terminal guanosine of viral mRNA is sufficient to satisfy the requirement for translation. Since the m^{7}G is in pyrophosphate linkage through its 5′-OH to the adjacent nucleotide[1], it contains free 2′, 3′-hydroxyl groups and can be removed after periodate oxidation by β elimination with aniline[7,11]. The effect of the removal of m^{7}G on reovirus mRNA activity was tested as follows. Reovirus mRNA was synthesised *in vitro* in the presence of SAM. It contained a mixture of molecules with 5′-terminal m^{7}GpppG^{m} (75%) and ppG (25%) (our unpublished results). After sequential treatment of the mRNA with periodate and aniline[7,11], the extent of removal of the 5′-terminal m^{7}G was 87% as determined with ^{3}H-methyl-labelled mRNA synthesised and treated in parallel. Since the translation of methylated reovirus mRNA is unaffected by SAH[5], the relative messenger activities of β-eliminated and untreated mRNAs were compared in wheat germ extracts containing SAH to prevent methylation of the 5′-terminal guanosine during protein synthesis. As Fig. 4 shows, β-elimination of premethylated mRNA resulted in the loss of about 85% of the mRNA activity compared with removal of most of the 5′-terminal m^{7}G. This loss of activity was not caused by chemical degradation of the RNA since the sedimentation profiles of the β-eliminated and untreated mRNAs in glycerol gradients were identical and included the l, m and s classes of mRNA[3].

Furthermore, the messenger activity of the β-eliminated RNA which consists of a mixture of molecules with 5′ termini of the types: (*a*) pppG^{m} (87%×75% = 66% of the total), (*b*) m^{7}GpppG^{m} (9%) and (*c*) ppG (25%), was partially restored when SAM was included in the wheat germ extracts (Fig. 4). The results indicate that chemical treatment did not irreversibly inactivate the mRNA. If, as suggested by these results, m^{7}G is

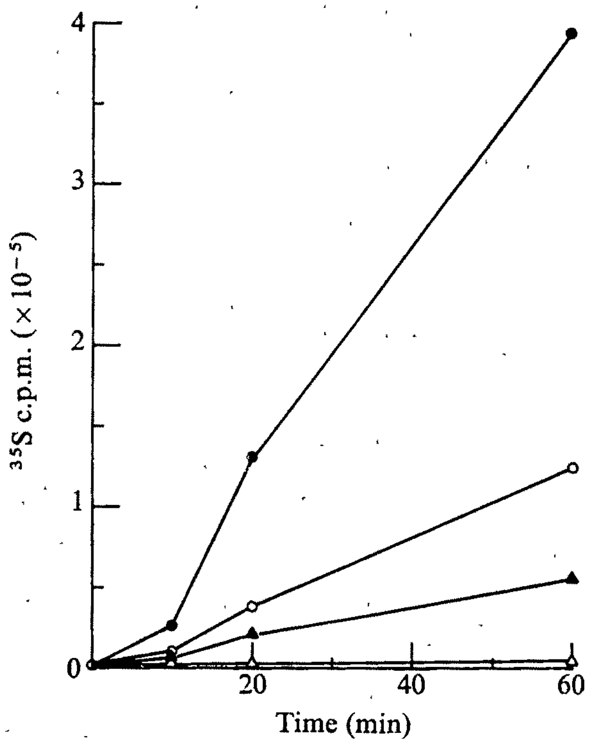

Fig. 4 Effect of removal of 5′-terminal m^{7}G on the ability of reovirus mRNA to stimulate protein synthesis. Methylated mRNA was synthesised *in vitro* with purified reovirus[3]. A sample of this RNA was oxidised with 10 mM potassium periodate, and the 5′-terminal m^{7}G was removed by incubation for 2 h at room temperature with 0.33 M aniline[11,12]. Equal amounts of untreated and treated RNA were used to programme polypeptide synthesis in wheat germ cell-free extracts as described[5]. Incorporation of ^{35}S-methionine into acid-precipitable material was determined. Untreated mRNA in the presence of 160 μM SAH (●); β-eliminated mRNA with 160 μM SAH (▲) or 2 μM SAM (○); and no added mRNA (△).

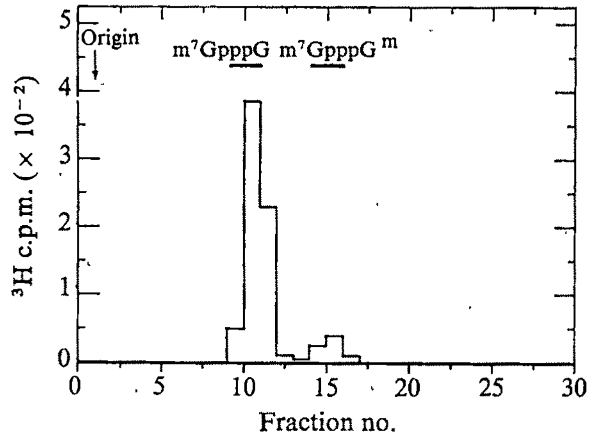

Fig. 5 5′-Terminal structure of β-eliminated reovirus mRNA after methylation by wheat germ extract. β-Eliminated reovirus mRNA was incubated for 20 min in conditions of protein synthesis in wheat germ extract with methyl-^{3}H-SAM (2 μM, 7.3 Ci mmol^{-1})[8]. The methylated RNA was isolated by phenol extraction and glycerol gradient centrifugation as in Fig. 1. After digestion with *Penicillium* nuclease and alkaline phosphatase, the enzyme-resistant ^{3}H-labelled material was analysed by paper chromatography[1]. The marker compounds were purified by electrophoresis of *Penicillium* nuclease plus phosphatase digests of reovirus mRNA methylated during transcription by virions (m^7GpppG^m) or during protein synthesis by wheat germ extract (m^7GpppG).

essential for translation of viral mRNA, restoration of the messenger activity of β-eliminated mRNA should be accompanied by the formation of new 5′-terminal m^7G. To test this possibility, treated mRNA was incubated with the wheat germ extract in the presence of ^{3}H-methyl-SAM in conditions used for protein synthesis. Radioactivity was incorporated into the RNA, and analysis by the procedures described in Fig. 1*a* revealed that all the ^{3}H was present in 5′-terminal structures. To determine which types of 5′ termini are substrates for methylation by the wheat germ extract, the ^{3}H-labelled mRNA was isolated from the extract after protein synthesis, digested with *Penicillium* nuclease and alkaline phosphatase and analysed by paper chromatography (Fig. 5). About 95% of the radioactivity migrated with m^7GpppG. The 5′-terminal material was further characterised as the monomethyl structure by nucleotide pyrophosphatase and phosphatase digestion followed by electrophoresis as in Fig. 3. The remaining radioactivity (5%) migrated in the position of m^7GpppG^m, indicating that only a small fraction of the predominant type of mRNA molecules, that is those with 5′-terminal pppG^m, can be methylated and blocked by the wheat germ extract. The results demonstrate that the recovery of messenger activity of β-eliminated RNA is paralleled by the formation of new 5′ termini containing m^7GpppG from molecules with 5′-terminal ppG. Since molecules containing 5′-terminal ppG comprise only one-fourth of the total mRNA, this finding is consistent with the incomplete restoration of the activity of β-eliminated mRNA.

5′-m^7G in reticulocyte mRNA

Globin mRNA, like reovirus RNA[12], is resistant to 5′-terminal labelling by the polynucleotide kinase procedure (R. Williamson, unpublished) suggesting that this cellular mRNA has blocked 5′ ends similar to those found in viral mRNAs. To test for the presence of 5′-terminal m^7G, rabbit reticulocyte mRNA was oxidised, reduced with ^{3}H-borohydride and analysed as in Fig. 1*a*. In addition to the 3′-terminal adenosine (520 c.p.m.), a peak of radioactivity (350 c.p.m.) was obtained in the position of presumptive 5′ termini (data not shown). Digestion of this 5′-terminal material with nucleotide pyrophosphatase and phosphatase converted all the radioactivity to the trialcohol derivative of m^7G (Fig. 6*a*). The results indicate that reticulocyte mRNA contains 5′-terminal m^7G in 5′ pyrophosphate linkage, that is with free 2′, 3′-hydroxyls. 5′-Pyrophosphate structures of this type are sensitive to β-elimination as shown for reovirus RNA[7,12]. If 5′-terminal m^7G is also required for translation of cellular mRNAs, its removal from reticulocyte mRNA by β-elimination should be accompanied by a loss of polypeptide synthesis in cell-free protein synthesising systems programmed by the treated mRNA. As Fig. 6*b* shows, after β-elimination, reticulocyte mRNA lost 80% of its capacity for stimulating protein synthesis. Treated and untreated mRNA coded almost entirely for a polypeptide which comigrated with globin during sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and its synthesis was reduced by80% following β-elimination of the mRNA.

A unique 5′-terminal structure of the type, m^7G(5′)ppp(5′)N^m has been found in various viral and cellular mRNAs including those of cytoplasmic polyhedrosis virus[13], reovirus[1,7], vaccinia[14,15], VSV[2], SV40[16], adenovirus (Y.F., A.J.S. and J. E. Darnell, unpublished), mouse L cells (Y.F., A.J. LaFiandra and A.J.S., unpublished results), monkey BSC-1 cells[16] and HeLa cells[17]. A common feature of these mRNAs is the presence of 5′terminal m^7G. The nature of the adjacent 2′-O-methylated nucleotides apparently varies. In addition, a series of low molecular weight RNAs from Novikoff hepatoma

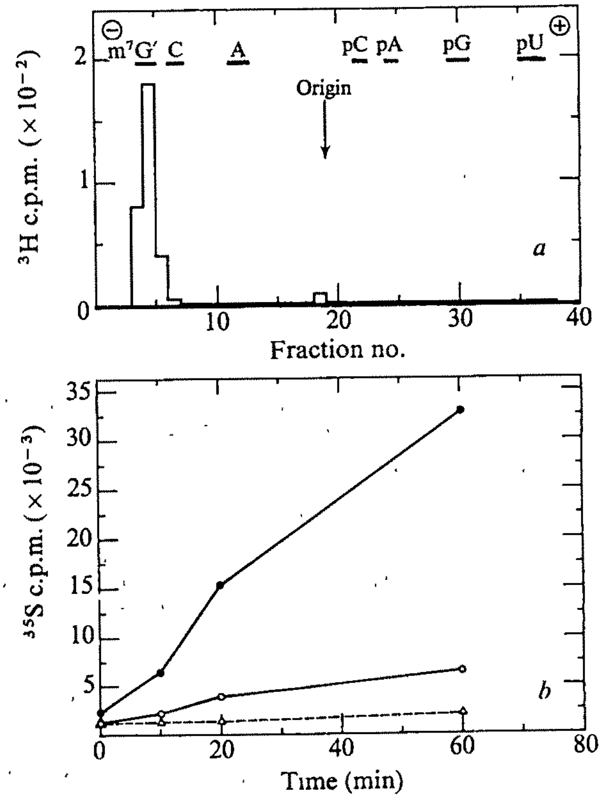

Fig. 6 5′-Terminal m^7G in reticulocyte mRNA and loss of translational activity after removal of m^7G by β-elimination. Reticulocyte mRNA was purified from cell lysates by phenol extraction and two rounds of binding to oligo(dT)-cellulose[21]. Aliquots of the eluted mRNA were used for labelling with ^{3}H-borohydride and for translation before and after β-elimination. *a*, mRNA was oxidised with 10 mM potassium periodate, reduced with potassium borohydride-^{3}H (10 mCi, 3.3 Ci mmol^{-1}), and digested with *Penicillium* nuclease and alkaline phosphatase. The presumptive 5′-terminal material was purified by paper electrophoresis, eluted, digested with nucleotide pyrophosphatase and phosphatase and analysed by paper electrophoresis. *b*, Equal amounts of mRNA (0.7 μg per 12.5 μl reaction mixture) were used to stimulate protein synthesis in wheat germ extracts before and after β-elimination as in Fig 4. Untreated mRNA in the presence of 160 μM SAH (●); β-eliminated mRNA in the presence of 160 μM SAH (○); no added mRNA (△).

cell nuclei have been found to contain 5′-terminal $m_3^{2,2,7}G(5')pp(5')N^mN^mN$, but their biological function has not been established[18]. Our results suggest that in addition to a possible role in mRNA processing[19], the 5′-terminal m^{7}G in mRNA is essential for translation. Unmethylated VSV and reovirus mRNAs, which are inactive in a wheat germ protein-synthesising system in the presence of SAH, become active when methylated[5]. Methylation occurs specifically in the 5′-terminal guanosine at the N^7 position. Removal of the m^{7}G by β-elimination of methylated mRNA results in a concomitant loss in the ability to stimulate protein synthesis. The presence of a 5′-terminal 2′-O-methylated nucleotide is not required for mRNA functions since the β-eliminated mRNA which contains 5′-terminal pppG^m is inactive and molecules containing m^{7}GpppG or m^{7}GpppA are active in protein synthesis. Reovirus mRNA synthesised in the presence of SAH contains 25% of the molecules with 5′-terminal GpppG (our unpublished results), and VSV mRNA made in the absence of SAM contains 5′-terminal GpppA (ref. 8). In each case, the mRNAs with blocked but unmethylated 5′ ends are inactive and become functional only after conversion to m^{7}GpppN. Reticulocyte RNA contains 5′-terminal m^{7}G and its removal by β-elimination results in the loss of its ability to stimulate polypeptide synthesis *in vitro*. It will be interesting to determine if 5′-terminal m^{7}G is a structural feature of all eukaryotic mRNAs that is required for a specific step in protein synthesis that is, ribosome binding.

We thank A. LaFiandra and M. Morgan for assistance. VSV mRNA was provided by A. K. Banerjee and D. Rhodes, and reticulocyte lysate was a gift from R. Gesteland.

Received March 3, 1975.

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Novel initiation of RNA synthesis *in vitro* by vesicular stomatitis virus

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The mRNA synthesised in vitro *by the virion-associated RNA polymerase of vesicular stomatitis virus contains the novel 5′-terminal structure G(5′)ppp(5′)Ap. . . . may be an intermediate in the formation of the 5′-termini of eukaryotic mRNAs.*

ALL messenger RNAs isolated from prokaryotes and from bacteriophages seem to be initiated by triphosphates at their 5′-termini[1-4]. In contrast, the 5′-terminal structures of natural eukaryotic mRNAs are believed to be different since they are resistant to phosphorylation by polynucleotide kinase after prior treatment with alkaline phosphatase[5]. The mRNAs synthesised *in vitro* by the RNA polymerases associated with the viral cores of several animal viruses including vaccinia virus[6], reovirus[7], vesicular stomatitis virus (VSV)[8] and silkworm cytoplasmic polyhedrosis virus (SCPV)[9], however, have either di- or triphosphates at their 5′-termini. Each of these viruses has been shown to possess an RNA methylase activity which transfers methyl groups from S-adenosyl-methionine (SAM) to the 5′-termini of mRNAs during their synthesis *in vitro*[10-13]. Furthermore, these methylated mRNAs all contain at their 5′-termini a blocked structure consisting of 7-methylguanosine linked by a pyrophosphate bridge to a 2′-O-methylated purine, having the general form $m^7G(5')ppp(5')Pup^m$. . . (refs 14–16). In the case of VSV the 5′-terminal structure is $m^7G(5')ppp(5')Ap^m$. . .[17].

During investigation of the mechanism of formation of this unusual structure, we discovered that in certain conditions, the virion-associated RNA polymerase of VSV synthesised RNA *in vitro* which terminated with a new type of structure, which was blocked but not methylated. This type of structure may be an intermediate in the formation of the 5′-termini of eukaryotic mRNAs.

Blocked 5′-terminal phosphates

The virion-associated RNA polymerase of VSV can be activated by non-ionic detergents to synthesise RNA products *in vitro* which are complementary to the genome RNA[18], contain poly(A) sequences at their 3′-termini[19] and are biologically active as messenger RNAs in a cell-free protein synthesising system[20]. Using Triton-disrupted VSV, we observed previously[12] that β,γ-^{32}P GTP was incorporated into the 5′-termini of the product RNA, but not β,γ-^{32}P ATP. This was unexpected since Roy and Bishop[8], using core particles derived from VSV by a polyethylene glycol-dextran phase separation procedure, found that most of the product RNA molecules contained 5′-pppA. VSV mRNA was synthesised *in vitro* using β,γ-^{32}P GTP as the labelled substrate, purified, and the 12-18S mRNA[21] was recovered as described in the legend for Fig. 1. After digestion with RNAase T$_2$ (which degrades RNA to 3′-nucleotides), the products were analysed by DEAE-cellulose chromatography as shown in Fig. 1. The major peak of radioactivity eluted at the position of tetranucleotides (−5 charge) and a minor peak (10–15%) at a slightly higher salt concentration. The presumptive 5′-terminal oligonucleotide contained in the major peak migrated to a position between the two mononucleotide markers, pG and pU, when analysed by paper electrophoresis (Fig. 2*a*). When digested with nuclease P$_1$ from *Penicillium citrinum*[22] (which degrades RNA to 5′-nucleotides) and alkaline phosphatase, all the ^{32}P-radioactivity was retained in a product

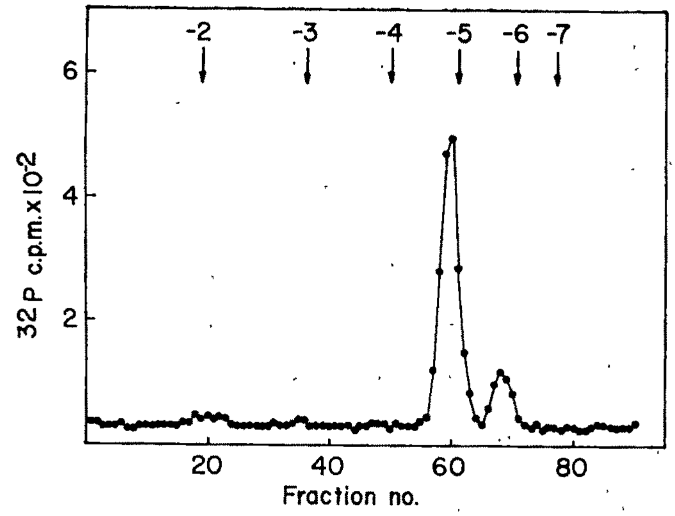

Fig. 1 DEAE-cellulose chromatography of the products of ribonuclease T_2 digestion of mRNA synthesised in the presence of β,γ-^{32}P-GTP. VSV mRNA was synthesised *in vitro* in an incubation mixture (0.4 ml) which contained 0.1 M NaCl, 0.05 M Tris-HCl (*p*H 8.0), 5 mM $MgCl_2$, 4 mM dithiothreitol, 1 mM ATP and CTP, 0.1 mM GTP and UTP, 63 μCi β,γ-^{32}P-GTP (3.7 Ci mmol^{-1}), 0.05% Triton N101, and 80–100 μg purified VSV. After incubation for 2 h at 30 °C, the reaction was terminated by addition of sodium dodecyl sulphate (SDS) to 0.5%. The product RNA was extracted with phenol, purified by Sephadex G-100 chromatography, and analysed by sedimentation for 17 h at 45,000*g* in a 15–30% (w/v) linear sucrose gradient containing 0.5% SDS[12]. The VSV mRNA species sedimenting between 12S and 18S (ref. 21) were pooled and precipitated with ethanol. The recovered RNA was digested with 2.5 U ribonuclease T_2 (Sankyo Co.) in 10 mM sodium acetate (*p*H 4.5) for 5 h at 37 °C. The products were eluted from a DEAE-cellulose column with a linear gradient of NaCl[23] and the radioactivity in each fraction was determined as Cherenkov radiation. Arrows indicate the elution positions of marker oligonucleotides expressed as their net negative charges.

which shifted to a position between the markers pA and pG (Fig. 2*b*). This result was consistent with the removal of an unlabelled phosphate group from the 3′-end of the oligonucleotide and showed that the ^{32}P-radioactivity derived from β,γ-^{32}P-GTP was completely protected from the action of alkaline phosphatase. On the other hand, the ^{32}P-radioactivity was entirely released as Pi when the 5′-terminal oligonucleotide (−5 charge) was treated with nucleotide pyrophosphatase, indicating that a pyrophosphate bond existed at the 5′-termini of the RNA molecules, possibly as $G_{(ppp)}X_p$, or $X_{(ppp)}G_p$, where X is an unknown base. The minor oligonucleotide peak eluting at −6 charge (Fig. 1) behaved identically when treated with the enzymes described above, and is believed to be a 5′-terminal fragment resulting from incomplete digestion by RNase T_2 (data not shown).

Structure of blocked 5′-termini

VSV mRNA was synthesised *in vitro* using α-^{32}P-GTP as the labelled substrate, and purified. The labelled RNA was digested with RNase T_2, and the products were analysed by DEAE-cellulose chromatography. Most of the radioactivity eluted as mononucleotides (−2 charge) but a fraction (0.24%) of the radioactivity eluted as a single peak at −5 charge similar to that shown in Fig. 1 (data not shown). This 5′-terminal oligonucleotide(s) was analysed further by paper electrophoresis following various enzymatic digestions as shown in Fig. 3. When treated with both nuclease P_1 and alkaline phosphatase (Fig. 3*a*) the radioactivity appeared as a single peak between pA and pG (compare Fig. 2*a*). When this material was treated with nucleotide pyrophosphatase, all the ^{32}P-radioactivity now comigrated with the pG marker (Fig. 3*b*). When alkaline phosphatase was added with nucleotide pyrophosphatase, all the radioactivity migrated as Pi (Fig. 3*c*). These results, together with that shown in Fig. 2, showed that the α- and β-phosphates of GTP were incorporated into the blocked structure, as $Gp^{\alpha}p^{\beta}$–pXp or Xp–$p^{\beta}p^{\alpha}$Gp.

Attempts to label the 5′-terminal oligonucleotide with α-^{32}P-CTP or α-^{32}P-UTP were unsuccessful. However, when α-^{32}P-ATP was used as the labelled substrate, a 5′-terminal fragment (−5 charge) similar to that obtained using α-^{32}P-GTP-labelled products, was recovered as described above. In contrast to the products labelled with α-^{32}P-GTP (Fig. 3*a*), 50% of the ^{32}P-radioactivity was released as Pi after digestion with nuclease Pi and alkaline phosphatase (Fig. 4*a*). This result showed that two phosphate groups from the α-position of ATP were incorporated into the −5 charged terminal fragment, and that one of them was 3′-terminal, being derived from ATP by nearest neighbour transfer. The other phosphate group was included in the blocked structure which migrated at the characteristic position between pA and pG (Fig. 4*a*). When the latter material was treated with nucleotide pyrophosphatase, all the ^{32}P-radioactivity comigrated with pA (Fig. 4*b*) and when treated with both nucleotide pyrophosphatase and alkaline phosphatase, all the radioactivity migrated as Pi (Fig. 4*c*). The pG and pA liberated by treatment of the putative 5′-dinucleotide with nucleotide pyrophosphatase (Figs 3*b* and 4*b*) were further confirmed to be authentic unmethylated pG and pA by paper chromatographic analyses

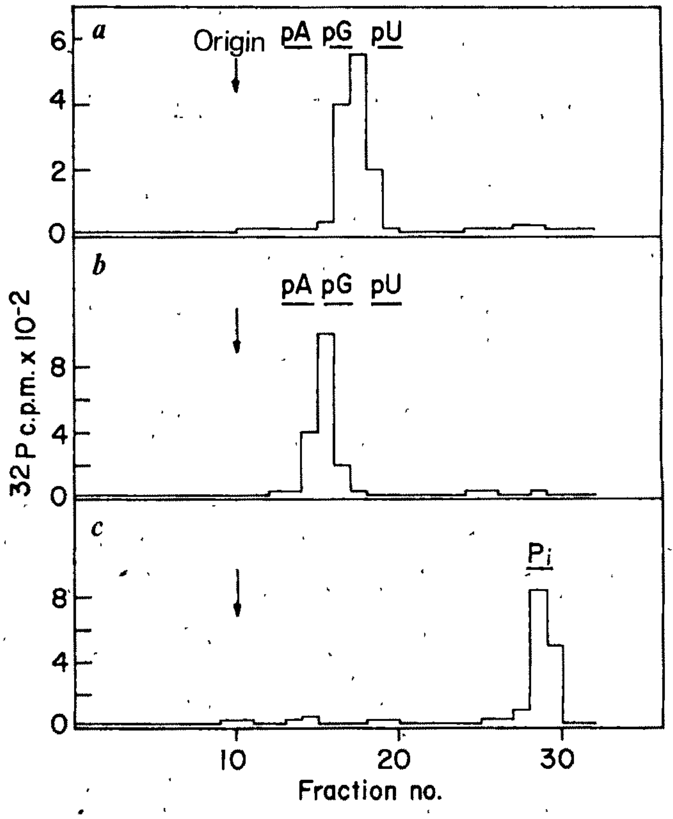

Fig. 2 Paper electrophoretic analyses of the products of enzymatic digestions of labelled RNA. The fractions containing the major peak of radioactivity eluting at −5 charge in Fig. 1 were pooled and desalted by dialysis. *a*, One portion was analysed directly by paper electrophoresis at *p*H 3.5[15]; *b*, a second portion was digested with nuclease Pi (a gift from Dr Kuninaka, Yamasa Co., Japan; 200 μg ml^{-1} in 10 mM sodium acetate, *p*H 6.0) for 30 min at 37 °C, followed by digestion with bacterial alkaline phosphatase (Worthington; 20 U ml^{-1} in 20 mM Tris-HCl, *p*H 8.0) for 30 min at 37 °C before analysis; *c*, a third portion was digested with nucleotide pyrophosphatase (Sigma; 0.05 U ml^{-1} in 20 mM Tris-HCl, *p*H 7.5, and 1 mM $MgCl_2$) for 15 min at 37 °C before analysis. Marker 5′-mononucleotides were included with the samples and their positions after electrophoresis were located by ultraviolet absorption. The paper was cut into 1-cm strips and the radioactivity in each was determined by scintillation counting.

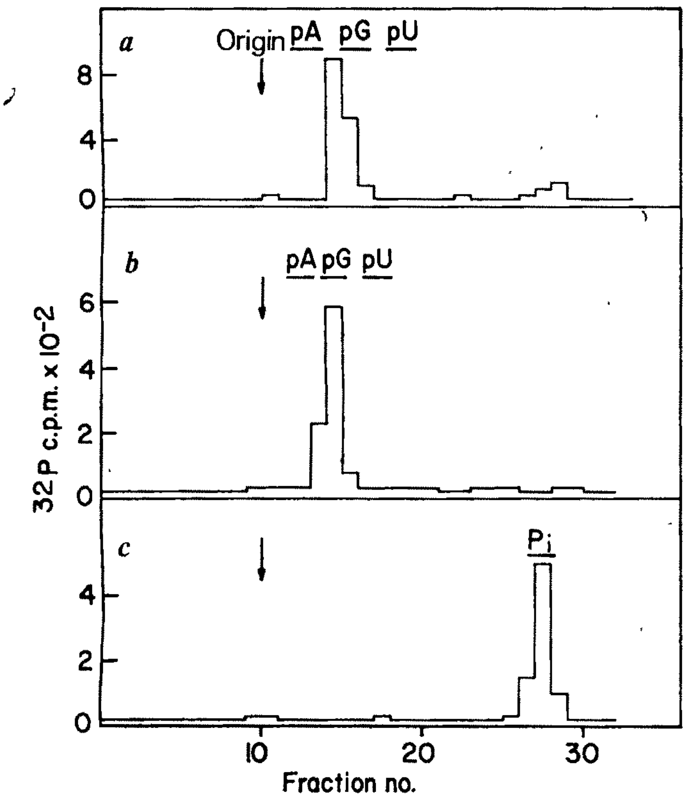

Fig. 3 Paper electrophoretic analyses of the products of enzymatic digestions of RNA labelled with α-^{32}P-GTP. VSV mRNA was synthesised and purified as described in the legend for Fig. 1 except that 50 μCi α-^{32}P GTP (18.5 Ci mmol^{-1}) was used as the labelled substrate. After ribonuclease T_2 digestion of the 12-18S mRNAs, the radioactive material which eluted from DEAE-cellulose at −5 charge (compare Fig. 1) was dialysed and digested with nuclease P_1 followed by alkaline phosphatase as described previously. *a*, This material was analysed directly by paper electrophoresis; *b*, the radioactive material from panel (*a*) was eluted from the paper, digested with nucleotide pyrophosphatase and a portion analysed directly; *c*, a second portion was digested with alkaline phosphatase before analysis.

(data not shown). From these results, the 5′-terminal structure of 12-18S VSV mRNA can be depicted as Gp$^{\alpha}$p$^{\beta}$–p$^{\alpha}$Ap$^{\alpha}$A . . . or Ap$^{\alpha}$–p$^{\beta}$p$^{\alpha}$Gp$^{\alpha}$A . . . .

Identity and orientation of blocking base

The appearance of the α-phosphates of both ATP and GTP in the blocked structure suggested that a 5′-5′ linkage was present, possibly as G(5′)ppp(5′)Ap . . . . If this structure does exist at the 5′-end of mRNA, the blocking base will contain free 2′- and 3′-hydroxyl groups and so will be susceptible to removal by periodate oxidation followed by treatment with aniline[24]. Accordingly, VSV mRNA synthesised using β,γ-^{32}P-GTP as the labelled substrate was purified and treated with potassium periodate followed by aniline. The resulting β-eliminated RNA was precipitated, digested with nuclease Pi, and analysed by paper electrophoresis (Fig. 5*a*). Approximately 65% of the ^{32}P-radioactivity comigrated with authentic ^{3}H-ATP marker, but not with GTP, which migrated 1 cm further. The remaining ^{32}P-radioactivity migrated as Pi and presumably resulted from trace amounts of contaminating phosphatases. The radioactive material that comigrated with ATP was eluted from the paper and digested with alkaline phosphatase before electrophoresis (Fig. 5*b*). All the ^{32}P-radioactivity was released as Pi, which indicated that the blocking group had been removed by β-elimination. As expected, the internal ^{3}H-ATP marker was converted to ^{3}H-adenosine by alkaline phosphatase. From these results we conclude that the 5′-terminal structure of the 12S–18S VSV mRNA synthesised *in vitro* is G(5′)ppp(5′)Ap . . . .

Possible intermediate in mRNA biogenesis

The results documented above illustrate that the virion-associated RNA polymerase of VSV synthesises mRNAs *in vitro* which contain a special type of modification at their 5′-termini. When RNA is synthesised *in vitro* using Triton-disrupted VSV, we observed: (1) no mRNA species are made which terminate with conventional di- or triphosphates, indicating that the blocking reaction, which renders 5′-terminal phosphates of RNA resistant to alkaline phosphatase, is complete; (2) in the absence of exogenous SAM no methylated nucleotides are detected at the 5′-termini, indicating that there is no endogenous methyl donor present in purified VSV and that addition of the blocking group does not require methylation; (3) when SAM is available during RNA synthesis the 5′-termini of all of the mRNA molecules are blocked and methylated[17]. Thus, it seems that in the VSV system there are two distinct enzymatic processes involved in the modification of the 5′-termini of the product mRNAs. An unmethylated, blocked terminal structure is formed first which then presumably is the substrate for subsequent methylation.

Using VSV ribonucleoprotein cores, prepared by a polyethylene glycol–dextran phase separation procedure, Roy and Bishop[8] reported that the RNA products synthesised *in vitro* were initiated with pppA . . . and pppG . . . . It seems likely that this fractionation procedure may have either removed or inactivated the blocking activity. Thus, it may be possible to isolate from VSV a separate enzyme which catalyses the blocking reaction. It has been reported that reovirus[7], vaccinia virus[6] and SCPV[9] synthesise RNA *in vitro* containing di- or

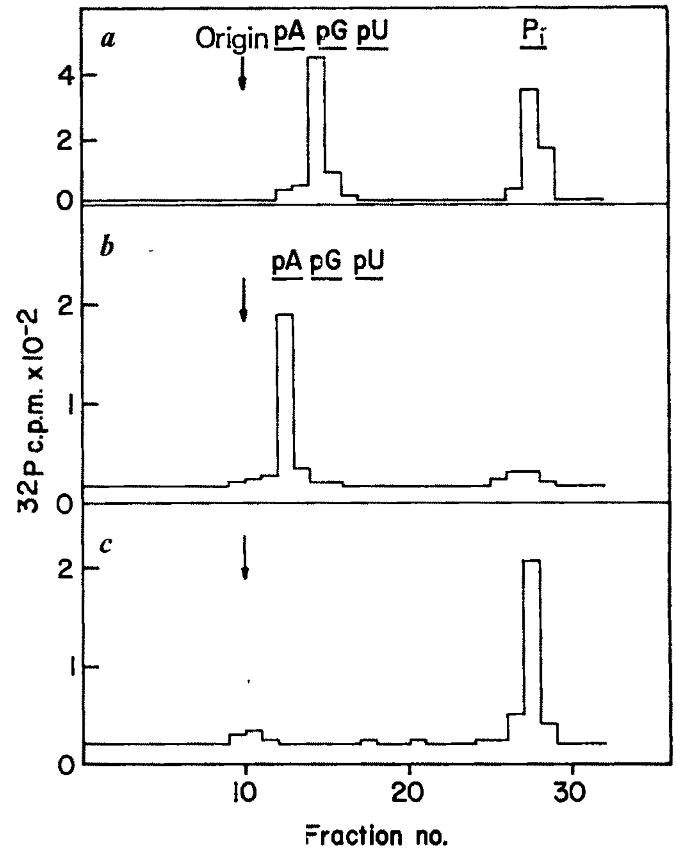

Fig. 4 Paper electrophoretic analyses of the products of enzymatic digestion of RNA labelled with α-^{32}P ATP. Experimental procedures were identical to those described in the legend for Fig. 3 except that 42 μCi α-^{32}P-ATP (15.7 Ci mmol^{-1}) was used as the labelled substrate. *a*, Analysis of the products after digestion with nuclease Pi followed by alkaline phosphatase; *b*, the radioactive material migrating between pA and pG in panel (*a*) digested with nucleotide pyrophosphatase; *c*, the same radioactive material from panel (*a*) digested with nucleotide pyrophosphatase followed by alkaline phosphatase.

triphosphates at their 5′-termini. In the presence of SAM, however, these same viruses can synthesise RNA which is both blocked and methylated[10,11,13]. It will be interesting to see whether unmethylated, blocked mRNA intermediates are synthesised *in vitro* by these viruses in suitable conditions. In fact, unpublished observations (S. Muthukrishnan and A. J. Shatkin, personal comminication) with reovirus indicate that approximately 20% of the mRNA molecules synthesised *in vitro* contain unmethylated blocked 5′-termini.

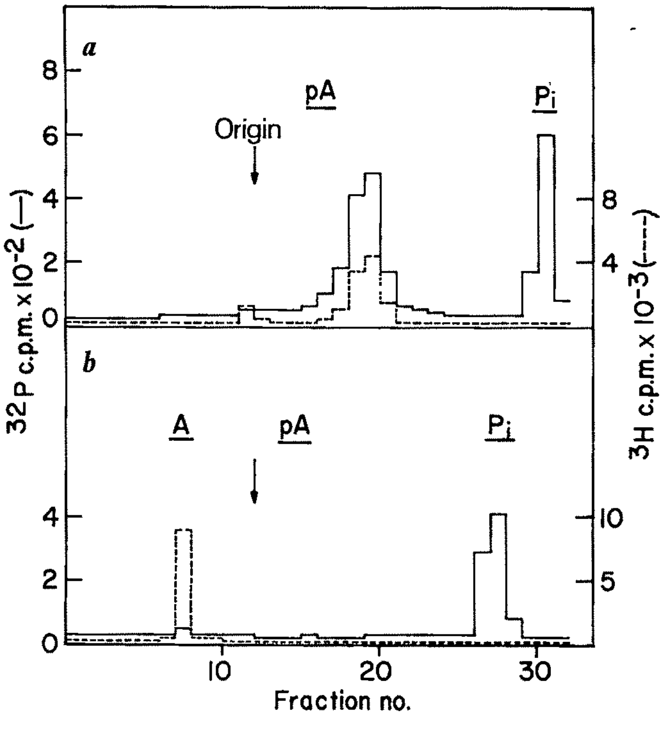

Fig. 5 Paper electrophoretic analyses of RNA products following β-elimination of β,γ-^{32}P-labelled VSV mRNA. VSV mRNA was synthesised and purified as described in the legend for Fig. 1. The 12S-18S mRNA species were oxidised with potassium periodate followed by treatment with aniline according to the method of Hunt[24], and the treated RNA was digested with nuclease Pi for 3 h. *a*, The products of this digestion were mixed with an authentic sample of ^{3}H-ATP and analysed by paper electrophoresis; *b*, the radioactive material which co-migrated with ATP in panel (*a*) was eluted from the paper and digested with alkaline phosphatase before electrophoresis.

Recent reports have indicated that eukaryotic mRNAs are methylated[25,26]. Moreover, methylated and blocked 5′-terminal structures of the form $m^7G(5')ppp(5')X^m$ are present in both HeLa and mouse L-cell mRNAs (Y. Furuichi and A. J. Shatkin, personal communication). Similarly, we have recently shown that the viral mRNAs isolated from VSV-infected cells also contain the same type of blocked and methylated structures[27].

These observations suggest strongly that the terminal modifications of mRNAs are biologically important events. The blocked but unmethylated 5′-terminal structure observed in the VSV system may be a general type of intermediate in the biosynthesis of eukaryotic mRNAs.

Received February 5, 1975.

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letters to nature

Origin of broad interstellar feature at 1.6 μm^{-1}

DIFFUSE interstellar absorption bands at 4,430, 4,760, 4,890 and 6,180 Å have been assigned to crystal-field transitions of octahedrally and tetrahedrally bonded ferric ions in an iron oxide[1-3]. The optical absorption spectra of terrestrial iron bearing minerals[4-6] often comprise, in addition to the crystal-field bands, a broad absorption (half-width 3,000 cm^{-1}) in the range 14,000–18,500 cm^{-1} (715–540 nm) marking $Fe^{2+}3dt_2 \rightarrow Fe^{3+}3dt_2$ intervalence charge transfer, the cations being located at the centres of adjacent edge-sharing octahedra. Here, I suggest that a discontinuity at ~ 1.6 μm^{-1}, which I see as a minor feature on the mean extinction curve[7] for several reddened stars, represents $Fe^{2+} \rightarrow Fe^{3+}$ charge transfer absorption in interstellar grains. A shallow emission feature observed at ~ 1.8 μm^{-1} in the reddening curve for another group of stars has been interpreted[8] as a dip lying between two absorptions centred at ~ 1.5 and ~ 2.4 μm^{-1}.

Extinction coefficients (ε) for $Fe^{2+} \rightarrow Fe^{3+}$ bands in silicates[4] are ~ 150 l mol^{-1} cm^{-1}, where ε is calculated from the band absorbance divided by the product of the specimen thickness (in cm) and the cation pair concentration (in mol l^{-1}). This value of ε is probably valid for oxides. The intense absorption of light by magnetite, Fe_3O_4 arises from a combination of $Fe^{2+} \rightarrow Fe^{3+}$ processes and crystal-field transitions ($\varepsilon \sim 500$; $f \sim 0.001$) in magnetically coupled ferric ions[9,10]. In magnetite[11], the 16-fold octahedral positions are occupied by equal concentrations of ferrous and ferric ions, and the 8-fold tetrahedral positions are occupied by ferric ions. Each octahedrally bonded ferrous ion shares four octahedral edges with adjacent Fe^{3+} centred octahedra. The Fe^{2+} concentration is 22.5 M, so the effective concentration of ferrous–ferric pairs is 90 M. The estimated absorption of the 1.6 μm^{-1} feature is 0.1 mag kpc^{-1}, corresponding to a thickness of magnetite along the line of sight of 0.03 μm. For a 1 cm^2 cross section of Fe_3O_4 over 1 kpc, the average density of Fe_3O_4 in the interstellar medium is therefore 1.6×10^{-26} g cm^{-3}. The iron density is then 1.2×10^{-26} g cm^{-3}, which is close to the currently accepted value [1] of 0.7×10^{-26} g cm^{-3}. This calculation shows that an oxide such as Fe$_3$O$_4$, with ferrous and ferric ions in adjacent edge-sharing octahedra, could be a component of interstellar dust.

The substitution of Ti^{4+} ions[12] into octahedral positions in magnetite will cause Fe$^{2+} \rightarrow$ Ti^{4+} charge transfer. This process is marked in terrestrial silicates by a broad absorption (half-width 5,000 cm^{-1}) centred in the 21,000–23,000 cm^{-1} (476–435 nm) region of the spectrum [6,13], with $\varepsilon \sim 10^4$ l mol^{-1} cm^{-1}. The Fe–Ti cosmic abundance ratio[14] is 250:1 so on a purely statistical basis the Fe^{3+}–Ti^{4+} ratio in octahedral positions in titaniferous magnetite is 80:1. The ε-values for the two processes suggest that if the Fe$^{2+} \rightarrow$ Fe^{3+} process is observable then the Fe$^{2+} \rightarrow$ Ti^{4+}, process could also be seen and it is possible that the latter process may be responsible for absorption in the region of the knee[15,16] in the extinction curve at ~ 430 nm.

I thank Professor N. C. Wickramasinghe and members of his staff for discussions.

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Received February 17; accepted March 25, 1975.

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Absorption of soft X rays by material within Gould's Belt

EARLY surveys of the soft X-ray diffuse background revealed an overall decrease of ~250 eV intensity from the galactic pole to the galactic plane[1-5]. The effect was attributed to absorption by interstellar gas and it was widely accepted that a substantial fraction of the flux originated from beyond the galactic disk. But the detection of emission from the plane of the Galaxy[2,3] (unit optical depth ~200 pc) and from the vicinity of a radio continuum feature, the North Polar Spur[6,7], demonstrated that at least part of the flux is galactic in origin. Recent surveys[8,9] provide greater sensitivity and reliability. The ~250 eV results have been combined in Fig. 1. From these data it has been shown that several regions of enhanced emission are associated with radio continuum or Hα features, and that there is no simple quantitative relationship between the soft X-ray intensity and 21-cm values of neutral hydrogen column density. It has been concluded on this basis that most if not all of the flux is generated within the Galaxy (refs 8, 9 and A. N. Bunner, unpublished). Since stellar sources or diffuse non-thermal processes cannot easily account for the observed intensity[8,10], thermal bremsstrahlung from a hot gas is now accepted as the most probable source mechanism. The discovery of a shallow OVI absorption line in the spectra of many nearby stars supports this hypothesis[11-13].

In spite of the lack of quantitative correspondence between the ~250 eV flux and regions of low HI column density, the Wisconsin workers note that enhancements are often observed where the neutral gas is lacking[8]. In addition the data of Fig. 1 exhibit a gross trend to smaller intensities at low galactic latitudes in agreement with the earlier surveys. If these effects are caused, at least in part, by interstellar absorption, there should be a general anti-correlation of soft X-ray intensity with material within one optical depth of the Sun. For 250-eV X rays this implies a correspondence with interstellar gas closer than ~200 pc.

The brighter stars visible to the naked eye and nearby reflection nebulae form a localised subsystem of the Galaxy inclined at ~20° to the galactic plane[14-16], which can also be detected by 21 cm observations of the local neutral hydrogen gas[17,18]. The best fit hydrogen equator[18] (Fig. 2) deviates by less than 4° from the original Gould's Belt. A comparison of Figs 1 and 2 shows that in the areas surveyed so far there is strong evidence for soft X-ray absorption by the local interstellar gas. The regions of low (~250 eV) intensity follow the curve of the local hydrogen equator and cover parts of the Scorpius–Ophiuchus complex at positive latitudes ($l^{\rm II}$ ranges from 330° to 30°) and the Orion–Taurus bulge in the southern hemisphere ($150° < l^{\rm II} < 195°$). But the most remarkable correspondence occurs between regions of low ~250 eV intensity extending away from the local hydrogen belt and several of the low velocity neutral hydrogen ridges[19] (Fig. 2). Although in the present data the spatial coincidence is not always exact, the reality of absorption by features of this type has been demonstrated by the results of a recent latitude scan in the Virgo–Hydra region (unpublished data by C.G.R. and others).

The interpretation of the absorption data is not straightforward because of the complex nature of the distribution of

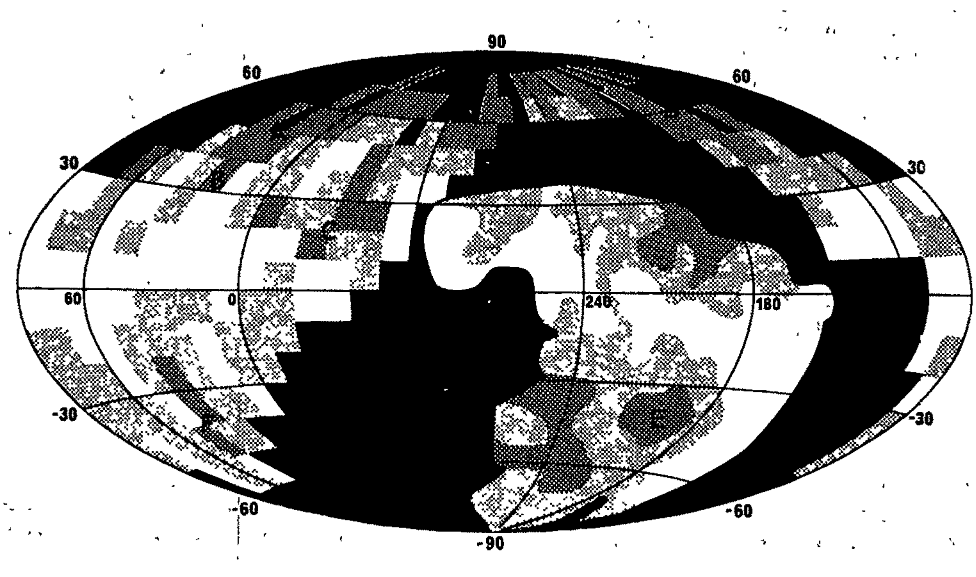

Fig. 1 The combined surveys of Williamson *et al.*[8] ($150° < l^{\rm II} < 290°$, $-90° < b^{\rm II} < 35°$) and De Korte[9] shown in galactic coordinates. Increased shading indicates increasing ~250 eV intensity except in the areas shown in black which have not been surveyed. The two intensity scales have been approximately normalised by comparing adjacent areas and by using the quoted brightness ranges. An area effected by scattered solar radiation has been removed from De Korte's survey[9] and the Vela–Puppis complex, visible in the original Wisconsin map[8], is not shown. Emission regions referred to in the text are labelled A–F.

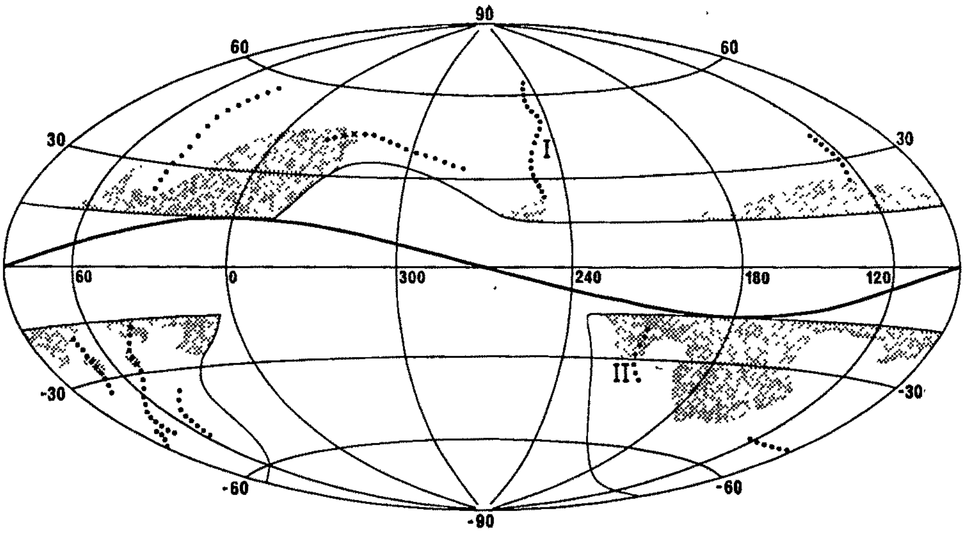

Fig. 2 The local neutral hydrogen equator taken from Goldstein and MacDonald[18] (thick line) with a representation of the extent of the major low velocity neutral hydrogen concentrations[19] (shaded) in the regions of sky for which data are available. The low velocity neutral hydrogen ridges of Fejes and Wesselius[19] are also shown (dots). The Sextans and Orion ridges are marked I and II respectively.

emitting and absorbing material. But the identification of enhancements A–F (Fig. 1) with radio continuum and Hα features [8,9,20,21] which are themselves closely interrelated[22] suggests that a combination of studies carried out over a wide range of wavelengths may result in a clearer understanding of the principal sources of emission. Large scale, ordered structures such as intersecting supernova shells[23] are of particular interest since the models of Spoelstra[24,25] show Radio Loops I and IV to intersect to form the first confirmed tunnel section. The radio continuum ridges and soft X-ray enhancements extending to high latitudes from region C in Fig. 1 may therefore be accounted for by emission from the tunnel wall.

Comparison of observed soft X-ray spectra with those derived from OVI results (which provide a value for the gas temperature) can provide distance estimates if values for the effective absorption cross section and average density of the intervening material are assumed. Approximate distances to several nearby emission regions can already be estimated from the data of Jenkins and Meloy[12].

Perhaps the most interesting absorption features shown in Fig. 1 are those associated with the neutral hydrogen ridges which often extend to intermediate or high latitudes. Since there is evidence that several of these ridges are associated with the radio loops[19], care must be taken when interpreting their modulation of the soft X-ray flux because they may merely separate regions of enhanced emission. The similarity of diffuse intensity on either side of the Sextans and Orion ridges (I and II in Fig. 2) suggests, however, that a genuine absorption effect is being observed. The 21-cm data and Brown and Gould coefficients[26] indicate that the additional gas path presented by each of these ridges is approximately one optical depth (63% modulation). So the observed modulations of ~20% (estimated from ref. 8) do not imply that a large fraction of the flux originates from beyond the ridges unless a significant unabsorbed local component of flux exists.

If it is assumed that the rather constant intensity observed in the plane of the local hydrogen gas represents such a component, the modulation of the remaining flux is ~50% and a substantial fraction of that flux would necessarily originate from beyond the ridges. Although the distances to the features are hard to estimate they probably extend as far as several hundred parsecs, and the existence of more distant regions of emission would imply an origin outside the galactic disk. It is difficult to see how such emission could be associated with a network of supernova tunnels unless some of these burst out of the neutral gas into a low density galactic halo. Although the present observations of the neutral hydrogen ridges can neither support nor rule out the existence of emission from beyond the galactic disk, the extended 250 eV enhancements at $l^{II} = 240°$, $-70° < b^{II} < -30°$ and in the Northern Hemisphere at $l^{II} \simeq 200°$ lie in the directions of the two deepest minima in the radio continuum sky[27] which coincide with regions of extremely low HI density. It may be difficult to account for these features by emission from within the Galaxy.

In conclusion it seems likely that the effects of soft X-ray absorption by material in Gould's Belt will contribute significantly to our understanding of the galactic sources of diffuse soft X-ray emission and may permit the uncertainty concerning the existence of a halo or extragalactic component of the flux to be resolved.

I thank Professor R. L. F. Boyd for encouragement and Drs J. L. Culhane and I. R. Tuohy for discussions.

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Received February 21; accepted March 27, 1975.

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Low frequency radio astronomy through an artificially created ionospheric window

THE ionosphere presents a nearly impenetrable barrier for ground-based decametric (3–30 MHz) radioastronomical observations, though a few valiant efforts have been made under special conditions to reach frequencies near 1 MHz (ref. 1).

To overcome this problem radio receivers can be placed on rockets and satellites[2-4]. There are, however, two basic problems with space radio observations. The first is the ambient plasma of the ionosphere, which at low frequencies interferes with the normal functioning of the antenna. The second is the very poor angular resolution available because the antenna rods used with these space vehicles are in general much shorter than the observing wavelength ($\lambda = 300$ m at 1 MHz).

Here we point out that the creation of an artificial ionospheric window to allow ground-based low frequency radio observations is now a realistic possibility.

Mendillo, Hawkins and Klobuchar[5,6] recently described the drastic depletion of the upper ionosphere (Fig. 1) brought about by the exhaust plume of the Saturn V rocket which placed NASA's Skylab Workshop into orbit on May 14, 1973. They concluded that the injection of molecular hydrogen and water vapour into the ionosphere increased dramatically the recombination rate of free electrons and oxygen ions in the F2 region.

Mendillo *et al.*[5] noted that, although the total electron content of the ionosphere decreased considerably, the critical frequencies for the E and F1 regions, as deduced from ionosonde measurements, did not change appreciably. The reason, they suggest, is that the ion–electron chemistry in these regions differs from that of the F2 region, and therefore the injection of H_2O and H_2 did not materially affect their loss rates. So it would be impossible to use this process during daytime to create a depleted plasma tube through the entire ionosphere.

During the night, however, the D, E, and F1 regions of the ionosphere disappear through natural recombination processes. The remaining night-time ionosphere, with a single peak around 300–350 km, owes its existence to the slow reaction rates of O^+ with O_2 and N_2 at these high levels. Consequently, a night-time injection of molecular hydrogen should greatly enhance the recombination rates at all heights and thus create a vertical tube of highly reduced plasma density through the entire ionosphere. This artificial ionospheric window should allow radio astronomical observations to be carried out from the ground between 1 and 6 MHz, and possibly at even lower frequencies.

The advantages of ground-based observations are several, for example calibration techniques are more reliable and antennas are free of plasma interference problems. The most important gain, however, might be the much improved directivity which could be achieved with simple interferometric techniques or with some of the already existing antenna arrays for long radio waves.

To take advantage of all this, one would have to create artificial ionospheric holes over existing antenna sites for low frequency radio observations. One long range possibility is to use the Space Shuttle, which NASA expects to have operational in the early 1980s and, in conjunction with the AMPS program, arrange to have an occasional hydrogen release over a low frequency radio observatory.

On the other hand, it seems logical to try to benefit from the present solar minimum when the ionospheric cutoff frequencies are at their lowest values. It seems, therefore, that for the next few years the best approach would be to launch rockets carrying a hydrogen payload from sites near suitable radio-astronomical facilities. These could be relatively small rockets able to carry a payload of about 100 kg to a height of about 300 km. As a first step it seems reasonable to use the 1,000-foot Arecibo telescope, which has a low frequency feed capable of operating at frequencies as low as 5 MHz (F. Drake, personal communication). The Arecibo observatory also offers the advantages of rocket launching facilities and continuous ionosonde monitoring of the ionosphere.

The height of the gas release should probably be about 50–100 km below the maximum of the night-time ionosphere, and preferably a continuous release over this altitude range. The reason is that the diffusion of H_2 through the ambient atmosphere strongly favours the upward direction[6], because of the exponential decrease of atmospheric density with height.

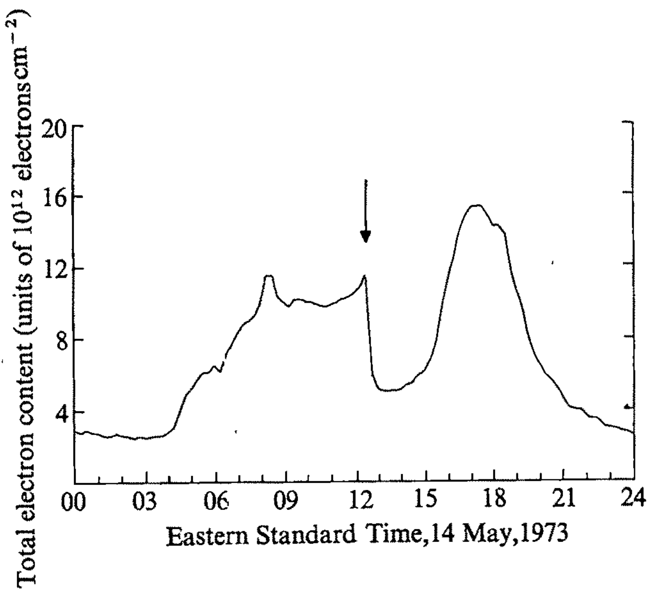

Fig. 1 Total electron content data obtained from the Air Force Cambridge Research Laboratory Sagamore Hill Radio Observatory in Hamilton, Massachusetts, looking toward the geostationary satellite ATS-3 on May 14, 1973. Arrow marks time of Skylab launch.

Using the simplifying assumption of spherical expansion in a uniform medium, the Skylab effect over a particular site could be accounted for by 1–2 s of the Saturn V exhaust,[5] that is, by a local injection of 40–80 kg of H_2 and 1–2 tons of H_2O. The release of 100 kg of hydrogen (3×10^{28} H_2 molecules), 50–100 km below the peak of the night-time ionosphere, should therefore create a vertical tube approximately 200 km in diameter with substantially reduced electron densities.

Considering this tube to have a length of 1,000 km (a volume of 3×10^{22} cm^3), such a release would produce an average density of about 10^6 H_2 cm^{-3}. The decrease of the electron density N with time at a given height is

$$dN/dt = -\beta N \qquad (1)$$

The recombination coefficient β is given by the expression

$$\beta = k\,[H_2] \qquad (2)$$

where $[H_2]$ is the concentration of hydrogen molecules and $k = 2\times10^{-9}$ cm^3 s^{-1} is the rate constant[7] for the reaction

$$O^+ + H_2 \rightarrow H + OH^+$$

With these values $\beta = 2\times10^{-3}\,s^{-1}$. This implies that the electron density near the peak of the night-time ionosphere ($\sim 10^5$ electrons cm^{-3}) would decrease to 5×10^3 in less than 0.5 h, and the density near 1,000 km ($\sim 10^4$ electrons cm^{-3}) would reach the same low level in less than 10 min. Of course, near the core of the tube the electron density should decrease much faster and to much lower levels, since the concentration of H_2, and therefore the value of β, would be much larger in this region.

We estimate that this tubular depletion should last for several hours. It would depend, among other factors, on the inclination of the Earth's magnetic field, which governs the diffusion of the surrounding plasma into the hole. The Skylab effect lasted 2–3 h, in spite of the fact that it occurred around local noon when the ionising radiation of the Sun is most effective.

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Received February 13; accepted March 18, 1975.

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Thermal regime of the Earth's interior

SEVERAL recent and apparently unrelated geophysical observations and deductions, when considered together, necessitate a fundamental re-examination of our ideas about the thermal state of the Earth's deep interior.

In summary they are:

(1) Precession must be discounted as a power source for the geomagnetic dynamo[1], leaving core convection as the only plausible basic mechanism. Therefore the temperature gradient in the outer core cannot be less than adiabatic.

(2) The thermodynamic Grüneisen parameter for the outer core is about 1.4 and is only slightly pressure dependent (R. D. Irvine and F.D.S., unpublished).

(3) The resulting adiabatic gradient is much steeper than the Fe melting point gradient[2] or the Fe–S eutectic gradient[3].

(4) Continents and ocean floors are both mobile, although the ocean floors are much more so[4], so that equality of the continental and oceanic heat fluxes which is observed[5] (in spite of gross differences between the radioactive constituents of the continental and oceanic crusts) cannot be explained in terms of an association of depleted regions of the mantle with continents.

(5) Hot spots, across which the tectonic plates move, are not themselves moving perceptibly with respect to one another and are presumed to mark features of a rigid lower mantle[4].

(6) A weak layer (asthenosphere) in the upper mantle allows virtually perfect isostatic balance of continent-sized blocks[6] but the lower mantle must have greater strength. Low degree harmonic features of the geoid are supported by stresses at depths of several hundred kilometres[7].

(7) Radiative transfer does not cause a dramatic increase in the thermal conductivity of mantle material at high temperatures[8,9].

As long as there remained a possibility that the geomagnetic dynamo is driven by precession[10], the fluid, outer core could be supposed to be stably stratified with a subadiabatic gradient. Although unconventional, the operation of a dynamo with the core in such a state could not be precluded[11] and the 'core paradox'[2,12] was avoided. This paradox arises because the adiabatic gradient in the core is much steeper than the melting point or eutectic temperature gradient, so that the conventional explanation of the solidity of the inner core as a pressure solidification of material from the outer core breaks down if the outer core is supposed adiabatic. But there now seems no possibility that precessional dissipation suffices for dynamo action. A 'rigid' core calculation indicated that the available power might be 3×10^{10} W (ref. 13), but Rochester[1] reported that proper inclusion of the internal core motions associated with core coupling to the mantle reduced the power estimate to less than 10^8 W, which certainly does not suffice to maintain the dynamo. Thus the temperature gradient of the outer core cannot be less than adiabatic.

So, how does one explain the apparently solid inner core? Elsasser and Isenberg[14] favoured an electronic phase transition in iron to the $3d^8$ state—collapse of all of the conduction or valence electrons into vacant 3d states. Probably because very little is known about such transitions (although one has been identified in caesium[15]) this suggestion has languished in obscurity; but now it emerges as the only real survivor. But it completely removes previously assumed constraints on the temperature of the core, based on the assumption that the inner core boundary represented the intersection of the temperature profile with the solidus of an iron-rich mix. Whether the inner core is really a crystalline solid must be doubted in view of its very high Poisson's ratio[16], which suggests some residual fluid character, but in any case extrapolation of ordinary melting point or eutectic data is irrelevant to an electronic phase transition.

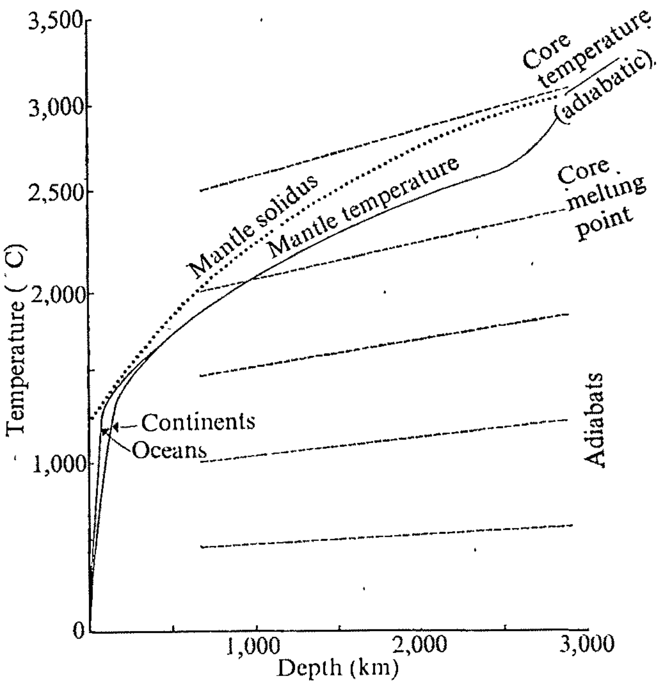
Rejection of core-constrained temperature profiles clears the way for an alternative approach, based on recent observations on the behaviour of the mantle. First, we note that the heat flux from the mantle into the continental crust is only about 0.015 W m^{-2}, the balance of the total geothermal flux (about 0.06 W m^{-2}) being provided by crustal radioactivity. But, since the continents ride about on rigid slabs of lithosphere, this mantle heat flux must be transmitted from depth by conduction. With an assumed conductivity of 2.5 W m^{-1} deg^{-1}, the required lithospheric temperature gradient extrapolates to temperatures of partial melting (~ 1,300 °C) at a depth of 150 km or so. This determines the thickness of the continental lithosphere, the more fluid asthenosphere below it being in convection. On the other hand the oceanic lithosphere is not a permanent feature. After formation at an ocean ridge, any section of it survives only for about 10^8 years before plunging back into the mantle at a subduction zone. If we suppose that its duration is simply its thermal relaxation (cooling) time τ, then with a thermal diffusivity representative of igneous rock, $\eta=1.2\times10^{-6}$ m^2 s^{-1}, we can estimate the thickness of the oceanic lithosphere as:

$$D=(\tau\eta)^{\frac{1}{2}}=70\text{ km}$$

It is important that these simple arguments give reasonable lithospheric thicknesses and are in accord with the equality of continental and oceanic heat fluxes without requiring any difference in radioactive heat generation in different parts of the mantle. Not only does it remove the implausible constraint to tectonic and convection theories that the continents must move about with their own sections of mantle, depleted in radioactivity, but also there is no reason for the temperature difference between the subcontinental and suboceanic mantle to extend into the lower mantle.

Increasing strength with depth below the asthenosphere (observation (6)) implies that the temperature profile diverges again from the solidus at greater depths. But we also require convective instability of the lower mantle to remove heat from the core, which, with a reasonably constrained conductivity[17], cannot be less than about 3×10^{12} W (ref. 18). In the case of the

Fig. 1 Suggested temperature profile of the mantle.



mantle, which has both a lower temperature and a smaller Grüneisen parameter, ~ 0.9 (R. D. Irvine and F.D.S., unpublished), than the core, we can hardly avoid the conclusion that the solidus gradient is steeper than the corresponding adiabat, at least in the upper levels, so that there is no difficulty in accepting an actual temperature gradient between the two. This is precisely the condition required for the establishment of narrow convective plumes[19]. The temperature gradient in the lower mantle gives increasing strength with depth, inhibiting convection in the whole mantle, but core heat must melt or nearly melt a thin layer at the base of the mantle. As this melted layer tends to rise, it finds that it is in a super-adiabatic gradient and so is convectively unstable, but that as it rises its melting point falls more rapidly than its actual temperature (decompression being approximately adiabatic) so that it becomes increasingly fluid and therefore heats a narrow channel to guide subsequent flow.

These considerations are summarised by the illustrative temperature profile in Fig. 1. This curve can be extended into the outer core for any assumed depth of convection by taking the adiabatic gradient (for which the assumption of a constant Grüneisen ratio of 1.4 suffices), but the required heat sources may be distributed through the outer core only, so that eventually we must reach a depth at which the heat flux is insufficient to support the adiabatic gradient. Below this the heat flow is diffusive only, but detailed calculations[20] show that the depth of transition from convective to diffusive regimes is very sensitive to the total heat flux; a 10% increment above the flux required just to maintain diffusion down the adiabatic gradient causes convection over most of the outer core. Thus it is reasonable to assume an adiabatic gradient through the whole outer core. This gives a temperature ratio between the inner core boundary and core–mantle boundary, $T_{IC}/T_{C-M}=1.33$ and, with the values in Fig. 1, an inner core temperature of 4,100 °C.

It may be noted that the necessity for 3×10^{12}–5×10^{12} W of radiogenic heat in the core seems unavoidable. Thus the geophysical requirement for potassium in the Fe–S outer core seems even stronger than the geochemical evidence[21-23] for it.

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Received February 20, accepted March 25, 1975.

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Wire strainmeters on ice

We have installed a geophysical wire strainmeter for brief periods on the Roslin glacier, Stauning Alps, East Greenland and on the sea ice at Bylot Sound, Thule, North-west Greenland. The technique promises to yield information concerning the mechanism of deformation and flow in ice.

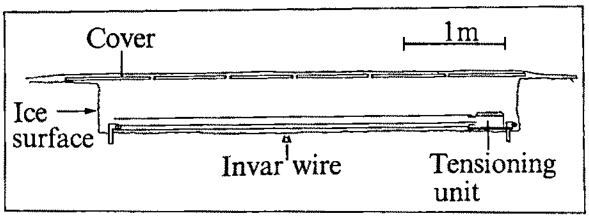

Fig. 1 The strainmeter installed on the glacier.

Previous studies of ice flow have involved intermittent measurements of surface markers[1], but although such methods illustrate the presence and rate of cumulative motion over large distances they do little to reveal the internal mechanism of flow. Few measurements have been made of the detailed strain behaviour of ice continuously at a point *in situ*[2,3]. For example, it is not known whether glacier flow is episodic and rapid with long periods of quiescence, or whether continuous creep occurs.

The wire strainmeter used in the experiments was designed for studies of earth strain (ref. 4 and G. C. P. King and R. G. Bilham, to be published) and has a measuring range of 10^{-4} strain (Fig. 1). The strainmeter uses 0.5-mm Invar wire held in tension by a lever and weight system. Rotation of the tensioning lever is detected electronically, amplified, filtered and recorded on a chart recorder. Using it as a 10-m strainmeter results in a maximum strain resolution of 10^{-10} in an environment where the temperature is stable. The temperature coefficient of the instrument is approximately 5×10^{-7} per °C. The instrument weighed only 10 kg and was easily transported to the sites.

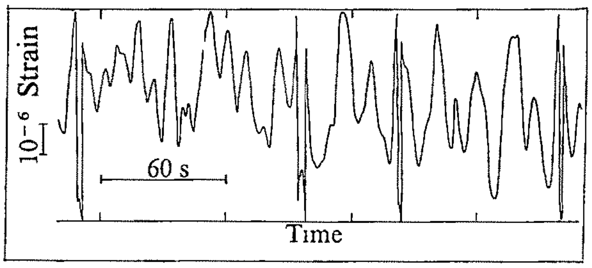

Fig. 2 Continuous record of strain changes measured on the sea ice in Bylot Sound, North-west Greenland in June 1974. The abrupt changes are d.c. shifts automatically introduced to keep the record on the chart.

The instrument was connected to the ice using two ice screws—a quick and simple means of obtaining the attachment stability required. Temperature stability (0 ± 0.5 °C) was obtained by building a cover when the strainmeter was on the sea ice and digging a 0.5-m trench and covering it when the strainmeter was installed on the glacier.

In June 1974 the instrument was installed on some first-year ice in Bylot sound (76°27′ N, 69°30′ W). The ice there was 0.86 m thick 71.7 m from the ice edge and 140 m from the shore. Some of the data obtained are shown in Fig. 2. Figure 3 is a frequency transform of approximately 1 h of data. The high-frequency part of the spectrum is suppressed by electronic filtering (−3 dB at 6-s periods). The spectrum is similar to that obtained from sea waves[5] with a dominant peak at approximately 16 s. There is a pronounced minimum between periods of 1 and 2 min, and the waves of longer period (20 min) present probably arise from seiches in the bay[6].

In July and August the instrument was taken to the Roslin glacier (71°51′N, 24°59′W). The glacier is a typical sub-polar–valley glacier 33 km long, 2 km wide and 300 m deep at the site. The surface velocity at the site, determined from four years of stake measurements, is about 9.5 m yr⁻¹. The instrument was installed centrally, parallel to glacier motion, slightly below

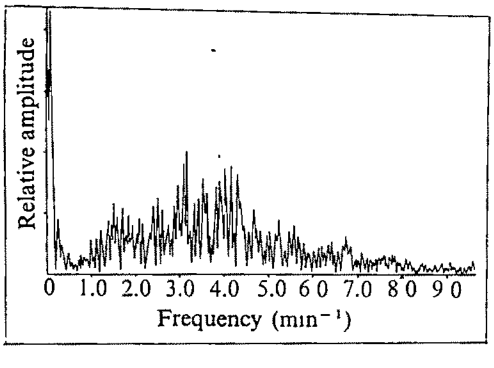

Fig. 3 Frequency spectrum of approximately 1 h of data obtained from the strain changes observed on the sea ice. The large peak on the left is probably due to seiches in the bay.

its junction with the Dalmore glacier and just below the firn line. Approximately three weeks of data were obtained with a chart speed of 18.8 cm h^{-1}. The records show long periods of slowly varying strain (10^{-7} a week) which may be partly instrumental drift, with occasional rapid, smooth excursions which recover after several minutes. A representative event, possibly indicating the transient strain field from a propagating creep event within the glacier is shown in Fig. 4. The temperature was monitored continuously and showed no such fluctuations. An array of strainmeters would make it possible to test this hypothesis.

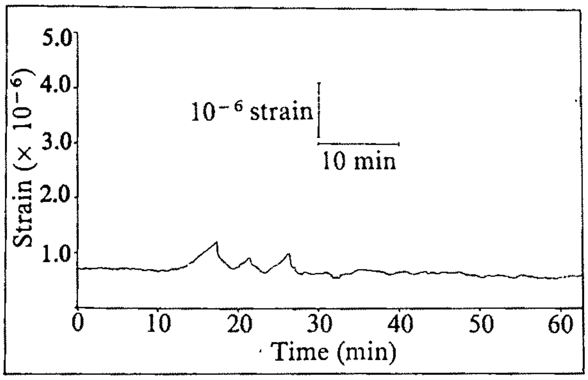

Fig. 4 Creep event observed on the strain record of a wire strainmeter on the surface of the Roslin glacier, East Greenland in August 1974.

The two preliminary experiments have convinced us that further studies with wire strainmeters on ice are justified. The study of creep processes in ice may have relevance to other disciplines where *in situ* deformation is slower, for example studies near geological faults[7].

Part of this work was funded by the Royal Society. D. J. G. is in receipt of a postgraduate award from the Science Research Council.

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Late Cretaceous and Palaeogene palaeolatitudes of the Ontong Java Plateau

THE Pacific plate has undergone a substantial northward displacement during the late Mesozoic and the Cainozoic. Here we give additional documentation for such motion based on palaeomagnetic measurements of a sequence of sedimentary and basalt samples collected from middle Oligocene to Aptian sections of Deep Sea Drilling Project (DSDP) site 289 (ref. 1; 00° 29.92′S, 158° 30.69′E) drilled on the Ontong Java Plateau.

The plateau is a major submarine feature more than 1×10^6 km^2 in area. It consists of an unusually thick crustal section overlain by approximately 1 km of flat-lying calcareous sediments[2]. We investigated site 289 mainly to determine the latitude of formation of the plateau, so our present results pertain only to the older site 289 cores. Initially, in addition to not knowing the extent of drilling disturbance within the sections, it was uncertain whether meaningful palaeomagnetic results could be obtained from such highly calcareous sediments. Palaeolatitudes determined from the measured palaeomagnetic inclination values of these samples, however, not only yielded an indication of the latitude of formation of the plateau but also provided a partial record of its subsequent northward movement during the interval from about −110 Myr to about −30 Myr. Other palaeolatitude trends measured in sediment samples from DSDP sites have also been attributed[3–5] to plate motion.

Ages were determined from the biostratigraphic report[1] by interpolating between well known planktonic foraminiferal datum planes[6]. The oldest core, core 30–131, reached upper Aptian sediments containing the foraminifer *Hedbergella trochoidea*. In keeping with current calibrations of Cretaceous stage boundaries[7], an age between about 108 and 106 Myr is assigned to the lowermost sediment samples.

We do not yet have evidence with which we can firmly establish the age of the basalt, and thus determine whether there is a hiatus at the basalt–sediment contact. Initial radiometric age determinations resulted in dates of −85±10 and −77±9

Fig. 1 Palaeolatitudes plotted against time for samples from DSDP site 289, Leg 30 (00°29.92′S, 158°30.69′E). Intensity of magnetisation of the sediment samples was generally of the order 1×10^{-7} gauss. But the angular difference between multiple measurements of single samples at the 100-oersted level typically did not exceed 2°. ●, Sediment; ■, basalt; ▲, mean of basalt.

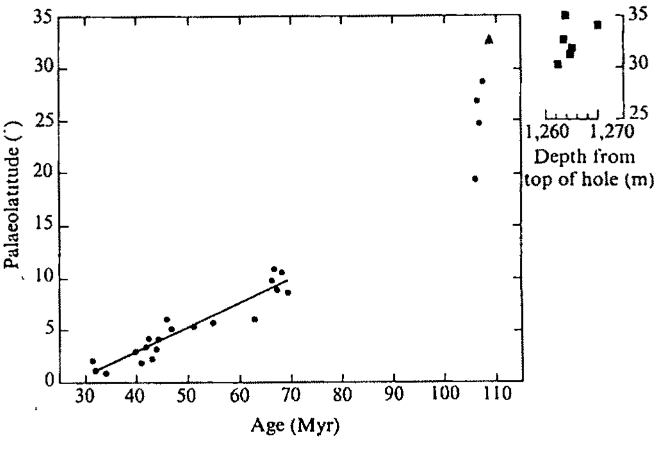

Table 1 K–Ar age results from the basalt, DSDP Hole 289, Core 132, Section 4, 141–143 cm

Subsample number	Weight (g)	Potassium (%)	Radiogenic argon ($\times 10^{-7}$ cm^3 g^{-1})	$^{40}Ar_{rad}/^{40}Ar_{total}$	Age±1 s.d. (Myr)
1	1.6269	0.0678 0.0757 0.0613	2.13	0.49	77± 9
2	2.8264	0.0650 0.752	2.36	0.69	85±10

The sample was holocrystalline with alteration confined to minor oxidation in the groundmass. The potassium content was determined by atomic absorption analysis; argon was released by induction heating, purified, and analysed using procedures of Keeling and Naughton[9]. An error analysis, similar to that of Cox and Dalrymple[10], indicated that the estimated analytical standard deviation arises almost entirely from uncertainties in the potassium measurements.

Myr for splits from one of the seven basalt samples (Table 1), but the conflict of these dates with the biostratigraphic ages for the oldest overlying sediment suggests strongly that they do not indicate the true age of the basalt. We tentatively assume the absence of an extensive basalt–sediment hiatus, and have therefore assigned an age of 109 Myr to the basalt.

Very little change in direction from that of the NRM was noted during stepwise alternating-field (a.f.) demagnetisation of each sediment sample[8]. Median destructive fields averaged about 300 oersted. There were no significant variations in demagnetisation characteristics throughout the sedimentary section, nor were there any visual signs of chemical alteration; further details pertaining to the palaeomagnetic data and techniques are reported elsewhere[8]. We attribute the tendency for palaeolatitude values to increase with age (Fig. 1) to relative motion between the region of the Ontong Java Plateau and a stationary planetary magnetic field. But the observed trends (Fig. 1) could be explained equally well by postulating a fixed lithosphere and a mobile field, or by motion of both field and lithosphere. Further, the present palaeolatitudes, determined assuming the Earth's field to be a geocentric axial dipole, would be in error by several degrees if the Early Cretaceous and Palaeogene field was, as has been proposed for the Neogene, not centred but offset into the Northern Hemisphere[11].

The slope of the least squares fit in Fig. 1 corresponds to an average rate of motion of about 2.5 cm yr^{-1} for the interval between about −70 and −30 Myr. The mean palaeolatitude anomaly[12] for the samples in this time span is consistent with this rate. The absence of data between about −105 and −70 Myr results from a late Aptian to late Campanian hiatus. But the palaeolatitudes for the oldest four sediment samples and mean palaeolatitude for six of the seven samples of basalt (one sample was excluded on the basis of its seemingly unstable remanence[8]) all lie above the best-fit line if it is extrapolated through the missing interval. This seems to indicate that the rate of crustal movement was more rapid during part or all of the time before −70 Myr. In turn, it is conceivable that the abrupt increase in palaeolatitudes reflected in the last four sediment samples merely represents a greater scatter of the data. This apparent scatter is, however, unrelated to biostratigraphic age control and is not the result of changes in sediment magnetic properties[8].

Because the ages of the youngest of our samples do not extend into latest Oligocene, the level reached by the known sediment-based polarity reversal sequence[13], only relative polarity can be defined from changes in the sign of the inclination. True polarity must, however, be determined in order to define the direction of motion of the lithosphere (northward or southward); our five oldest sedimentary samples are late Campanian, Albian, and late Aptian in age, all times of almost exclusively normal polarity[14-16]. It seems likely that these samples, and presumably also the basalt samples, are of normal polarity. They must have originated in the Southern Hemisphere, because their inclinations are all negative[8], and been displaced northward to the coring site.

Y. Lancelot and colleagues (unpublished) have determined rates and poles of rotation for the Pacific plate based on the northward shift, with increasing age, of equatorial sediment facies as observed in cores from DSDP sites, including site 289. Our rate of about 2.5 cm yr^{-1} between about −70 and −30 Myr is approximately half the northward rate predicted by their model for the site, but experimental errors in either method may be responsible for part of the disparity. The approximate latitude of formation we determine for the plateau (about 33° S), is in reasonable agreement with the approximately 40° S latitude suggested by Lancelot and colleagues.

Although at present we lack palaeomagnetic data necessary to describe the Neogene tectonic history of the Ontong Java Plateau, such results are available for the Central Pacific Basin, located east of the plateau and separated from it by the southern extension of the Marshall–Gilbert–Ellice Islands Ridge.

Palaeomagnetic evidence from deep-sea cores from the Central Pacific Basin indicates that the basin underwent continual northward displacement throughout the past 30 Myr at a rate of about 7 cm yr^{-1} (ref. 17). Unpublished work by Lancelot and colleagues, and other stratigraphic evidence for the migration of the zone of maximum sediment accumulation as observed in central Pacific DSDP cores[18], confirm the northward displacement of the basin. But those models (Lancelot and colleagues, and ref. 18) suggest motion at a much slower rate than our postulated 7 cm yr^{-1}.

We thank C. E. Helsley for use of the cryogenic rock-magnetometer at the University of Texas at Dallas, R. Moberly for comments, and V. A. Lewis for the potassium analyses. Supported by grants from the US Office of Naval Research and the US National Science Foundation.

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Received January 27, revised February 3, 1975.

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Dating a stalactite by electron paramagnetic resonance

OPTICAL and electron paramagnetic resonance (EPR) properties of natural and synthetic calcite (limestone, $CaCO_3$) have been studied extensively and it is known that ionising radiation in the form of cosmic rays or from radioactive elements introduces defects such as CO_3^- (a hole centre) and CO_3^{3-} (an electron centre), which are stabilised by impurities to form sources of thermoluminescence[1,2]. There are four thermal glow peaks above room temperature[3,4], some of which can be used to trace the history of calcite[5]. Here I report the presence of a radiation-induced defect in the growing stalactites of Japan's main calcite caverns and show that estimation of the accumulated defect concentration at several positions, using EPR, makes it possible to determine the age and growth rate of a stalactite.

Figure 1 shows EPR derivative spectra of the limestone at Akiyoshi karst plateau (Carboniferous to Permian) and of a stalactite at Akiyoshi, the biggest cavern in Japan. A prominent feature is the hyperfine sextet and the forbidden transitions ($\Delta m = \pm 1$) associated with the Mn^{2+} nuclear spin ($I = 5/2$). The EPR intensity of Mn^{2+} is much stronger in a limestone than in a stalactite, possibly because of the repulsion of Mn^{2+} in the recrystallisation of $CaCO_3$ during stalactite growth. Another feature of the EPR spectrum of the stalactite is the relatively intense signal with $g = 2.003 \pm 0.001$. The signal intensity is reduced by thermal annealing of the stalactite above 500 K, where thermoluminescence is observed. It is, however, also enhanced strongly when the stalactite is irradiated with ^{60}Co γ rays. Marshall *et al.*[1,2] observed that the CO_3^{3-} centre was stabilised at up to 500 K by a Y^{3+} impurity in the irradiated calcite crystal. The g tensors were $g_{xx} = 2.0012$, $g_{yy} = 2.0024$ and $g_{zz} = 2.0038$. The present g value of the polycrystalline calcite, and the thermal stability, agree within experimental error.

The effect of stresses caused by dynamic metamorphism[6,7], which also creates the ionisation and defects responsible for the high temperature thermal glow peak at 700 K in calcite, need not really be considered for a cavern stalactite; in any case the stalactite did not show any enhancement in EPR signal on grinding. The most plausible explanation for the presence of this radiation-induced defect in a stalactite is, therefore, natural radiation—cosmic rays and the radiation from radioactive elements such as ^{40}K, ^{238}U and ^{232}Th. Zeller used EPR to study the natural radiation damage of zircon, apatite and also carbonate, and observed a similar spectrum in limestone[5]. He also suggested the use of EPR for dating geological materials.

The observation that the EPR signal of $g = 2.003$ in a natural limestone (Permian; Neoschwagerina craticulifera Zone) is very weak (Fig. 1*a*) is puzzling, as the limestone has been exposed to natural radiation for about 2×10^8 yr. This is easily understood, however, in view of the fact that it was not possible to create this signal in the limestone by artificial γ irradiation. As the concentration of Mn^{2+} and other impurities such as Fe^{2+}, Si^{4+} and Al^{3+} in a limestone is very high, absorbed radiation energy (creating electrons and holes) would be consumed very efficiently at impurity sites at around room temperature.

Figure 2 shows the relative intensity of this signal as a function of the radial distance from the surface of a stalactite in the K branch of Akiyoshi-Do Cave. In every growing stalactite taken from four main caverns in Japan, the signal intensity at the surface is very small but increases linearly inside the stalactite. Since the surface of a stalactite is recent, and the calcite becomes older as one goes inside, this result clearly indicates that the EPR signal is associated with a defect formed by natural radiation and that the signal can be used for dating. The change in the signal intensity with radial position cannot be ascribed to the distribution of any specific impurity.

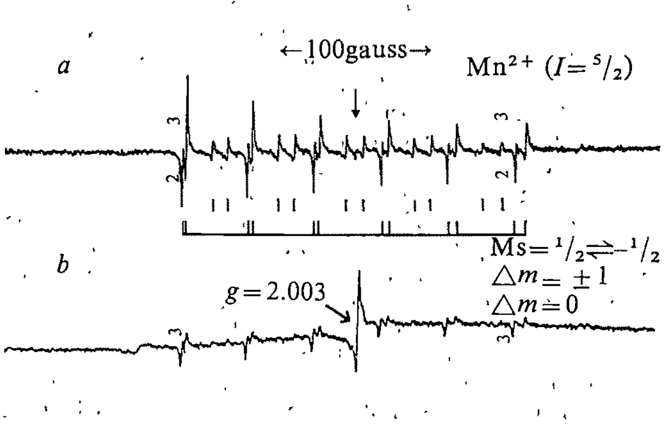

Fig. 1 EPR spectra of limestone (*a*) and a stalactite at Akiyoshi Cavern (*b*). The EPR hyperfine line sextet of Mn^{2+} ($\Delta m = 0$) and the forbidden transitions ($\Delta m = \pm 1$) are indicated in the figure. The signal with $g = 2.003$ (arrow) is associated with defects produced by natural radiation.

Table 1 Age and radial growth rate in time units of natural radiation dose (rad) for growing stalactites in Japan's main caverns

Cavern	Location (prefecture)	Terrestrial background radiation (m rad yr^{-1})*	Stalactite age (rad)†	Growth rate†‡ (μm rad^{-1})
Akiyoshi (Kurotani branch)	Yamaguchi (West Sanyo)	69	7×10^4	(*a*) 1.8 ±0.5 (*b*) 0.18±0.05
Hiraodai	Fukuoka (North Kyushu)	48	2×10^5	(*a*) 0.4 ±0.1 (*b*) 0.15±0.05
Ryusen	Iwate (East Tohoku)	32	3×10^4	(*a*) 1.5 ±0.5 (*b*) 0.8 ±0.3
Garashigama	Okinawa (South Island)	—	2×10^3	(*a*) 10.0 ±3.0

*γ-ray exposure rates arising from terrestrial radionuclides at 1 m above ground in each district[8]. Radiation damage would be caused mainly by α-rays from uranium and thorium in the stalactites, so this value is to indicate the geographical difference and is not a conversion factor for the actual age and annual growth rate.

†Time is expressed in rad, corresponding to the ^{60}Co γ-ray exposure to water. Actual age or growth rate must be obtained by dividing or multiplying by the annual radiation dose to the stalactite from both inside and outside. Estimation of actual age and growth rate is discussed in the text.

‡The growth rates determined are for the radial direction. The longitudinal growth rate is generally 8~10 times larger than the radial one. *a*, Radial growth rate of the stalactite close to the surface; *b*, radial growth rate inside.

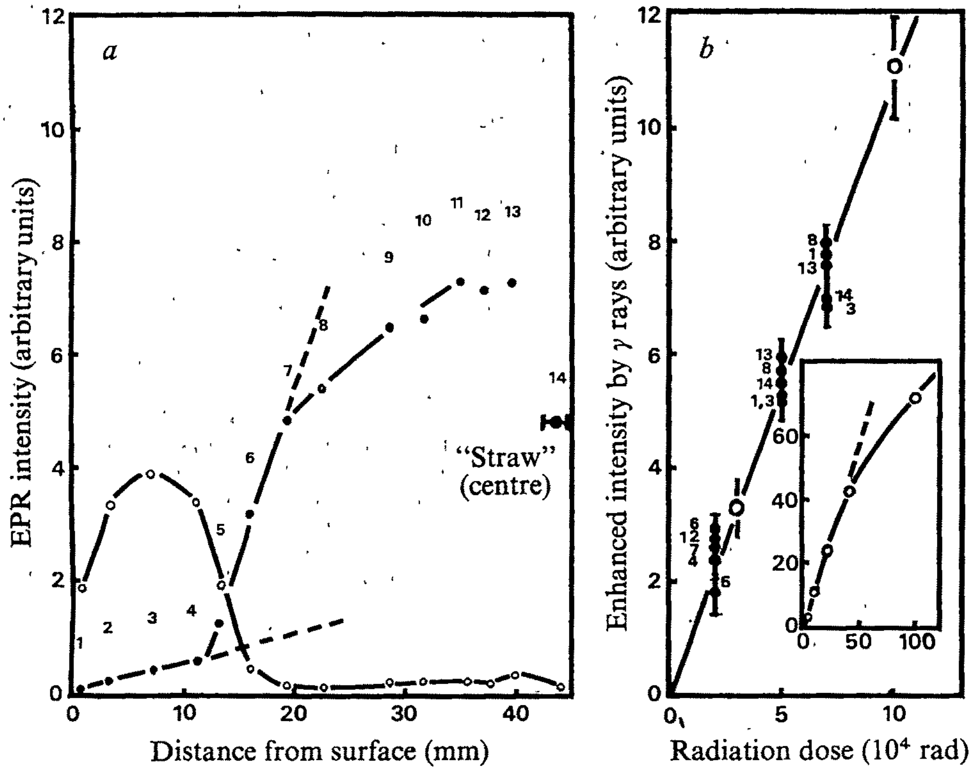

Fig. 2 *a*, Change in the EPR signal intensity as a function of distance from the surface of a stalactite at Akiyoshi. The signal intensity of Mn^{2+} impurity is also shown (○) ●, *g* = 2.003. *b*, Enhancement of the EPR signal by the exposure to γ rays from ^{60}Co for samples mixed (○) or taken from different positions (●). A straight line can be drawn (within ±10%) up to 10^5 rad. The EPR intensity is thus proportional to the dose of natural radiation, and so to the age, in this dose range. The stalactite's radial growth rates were deduced from the initial and subsequent slopes in *a* and the age was determined from the maximum signal intensity close to the centre "straw" position. The enhanced intensities of mixed powders are also shown in *b*.

Figure 2*b* shows the enhancement of the signal intensity brought about in samples from several parts of the stalactite, numbered 1 to 14, by exposure to ^{60}Co γ rays. The growth of the signal intensity is almost linear up to 10^5 rad and then deviates slightly from linearity. The growth rate above 10^6 rad is about half the initial rate and shows signs of saturation. The variations among samples from different locations in sensitivity to γ-ray exposure is within experimental error. Since the samples were usually taken within width bands of 1~2 mm, sensitivity differences arising from the microdistribution of impurites may be averaged out. As the signal intensity of the unirradiated stalactite corresponds to that observed after γ-ray exposure of ~ 10^4 rad, I simply assume linearity with the radiation dose. Thus, one can estimate the total dose of natural radiation received by a sample by comparing the signal intensity of the natural stalactite with the intensity as enhanced by γ radiation. Samples were kept in the dark for a few days after the exposure to γ radiation so that unstable defects could fade away. The signal intensity relative to that of Mn^{2+} was measured after irradiation. It was assumed that the signal intensity of Mn^{2+} is not altered by irradiation.

One can also estimate the age or annual growth rate of any part of a stalactite if the annual dose of natural radiation is known. Unfortunately, I do not at present know the radiation type or dose in Japan's caverns. Therefore, I show in Table 1 the age and growth rate on a time scale of the radiation dose (rad) for cavern stalactites. Terrestrial background γ radiation dose rates in the areas[8] are also given. The concentrations of U, Th and K in soils at West Sanyo (Yamaguchi area) was 1.3, 9.3 and 2.2 p.p.m., respectively; these values are higher than the averages for Japan (1.3, 5.6 and 1.4 p.p.m.). In East Tohoku, the concentrations are lower than average. The radiation source in the stalactite would be mainly these ions.

Aitken used 1 rad yr^{-1} as the annual dose rate for thermoluminescence dating of pottery[9] and if this value is used, the numerical values in Table 1 are the actual age and annual growth rate. This value would, however, be an overestimation for stalactites, as the U content is an order of magnitude lower than in pottery[10]. We are in the process of measuring the concentrations of these radioactive elements by γ-ray spectroscopy[8], and also the background radiation in Akiyoshi cave with a thermoluminescence dosimeter (TLD) of $CaSO_4$ (Tm) embedded in a stalactite.

Linear energy transfer (LET) effects on the radiation damage of a stalactite by α rays, by β rays and by γ rays of different energies must be considered. For example, the sensitivity of $CaSO_4$ TLD material to low energy photons is about 10 times that to ^{60}Co γ rays. On the other hand, the sensitivity to α or β rays is less than that to ^{60}Co γ rays, according to luminescence dosimetry studies. This holds good for $CaCO_3$, so the accurate estimation of the annual radiation dose for a stalactite is a topic to be treated separately.

The signal intensity at the centre ("straw") is about two-thirds that at a position close to the centre, as shown in Fig. 2. This is much more of a variation than that among samples taken from different distances. Polarisation microscope studies of a polished thin slice of a stalactite indicate that the *c* axis of the calcite crystals is almost in a radial direction, except in the centre ("straw") position, where it is longitudinal. The results suggest that the centre part was made later, in agreement with the model of stalactite growth.

Figure 2*a* indicates that there is a distinct change in growth rate, the slow increase of the EPR signal near the surface indicating rapid growth of the stalactite. The same distinct change was observed in a stalactite at Hiraodai (Kyushu), very close to Akiyoshi (Yamaguchi). The change is, however, not as clear for stalactites at Okinawa and in northern Japan. The change in growth rate may be associated with a drastic change in the climate, which favoured the recent rapid growth.

The signal intensity increases linearly after this sharp change but saturates very close to the centre, either because of rapid growth there or because of an equilibrium between fading and defect formation. The annealing experiment indicates that part of this signal intensity decays with the thermoluminescence at 500 K. The centre is completely destroyed by 10 min of pulse annealing above 700 K. A fading time of about 3×10^5 yr is expected if the defect produced is related to the thermoluminescent glow at 600 K (refs 3 and 4). A stalactite also has a recently grown part, however, and this can be used for determination of the growth rate. Defects stable at higher temperature could be used for dating older parts of a stalactite, or limestone.

I thank Professor N. Itoh and Dr M. Ohta for their help and partial support of this research. I also thank Professors T. Higashimura and H. Shima for discussions.

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Trace elements in total particulate material from surface sea water

THE euphotic zone is an important marine environment in which the photosynthetic planktonic population of the oceans is in contact with inorganic solids. These inorganic solids, together with a proportion of the biomass, settle out and are eventually incorporated into marine sediments. There have been various investigations into the chemistry of marine plankton[1-3]. Little is known, however, of the elemental composition of the total particulate material from the euphotic zone on a worldwide basis. Because of this lack of data average trace element concentrations for particulate material from relatively large oceanic regions are useful because they give an indication of values to be expected in the euphotic zone in general. Here, we present preliminary data on the trace element composition of some total particulate material from the surface (0 to ~5 m) layers of the North Atlantic, South Atlantic, Indian Ocean and China Sea.

Samples were collected on board MV Cyclops during March and July, 1972, by pumping seawater from a position by the ship's bows (which minimised contamination from the ship's sides) through plastic tubing to a continuous centrifuge. Particulate material was retained on plastic liners which were stored at ~12 °C until the solids were removed by washing with distilled water in the laboratories at Liverpool. The particulate material was dried at ~40 °C, finely ground, and the trace elements determined spectrographically using an argon–oxygen atmosphere. The ship's track and the sampling positions are shown in Fig. 1, and the trace element composition of the particulate matter is given in Table 1 where it is averaged for each oceanic region. The trace element compositions of some marine sediments and plankton are listed in Table 2.

Table 1 Trace element concentrations in the surface particulate material (concentrations in p.p.m.)

Oceanic region*	Mn	Cu	Co	Ga	V	Ba	Pb	Zn	Mean: %organic carbon
North Atlantic (10)	145	74	11	4	38	191	52	159	24
South Atlantic (4)	85	52	16	3	69	72	72	260	18
Indian Ocean (6)	385	202	14	3	60	77	44	231	14
China Sea (7)	1,501	107	12	8	86	166	63	232	12
Overall average (27)	529	109	13	5	63	126	58	220	17

* Number of samples given in parentheses.

Total particulate material in the euphotic zone of the oceans consists of a mixture of continental weathering products (including organic and inorganic precipitates), authigenic inorganic precipitates, and organic components which include phytoplankton, detritus (dead organisms), zooplankton and bacteria. It is difficult to establish the trace element composition of any of the various components in this complex mixture because they cannot be completely separated from each other. Certain estimates can, however, be made by considering the average compositions of marine sediments and plankton. Continental weathering products brought to the oceans by river run-off have a variety of particle sizes. In general, the coarser material will tend to settle out close to the continental margins and, to a first approximation, its composition should be similar to that of the average near-shore sediment. On average, the particulate material is deficient in Mn, Ga, V and Ba, and is enhanced in Cu, Pb and Zn relative to near-shore sediments (Table 2). Finer particles of a continental origin can escape deposition on the shelf regions and be transported to pelagic areas by current systems in the upper water layers These particles, which include iron and manganese oxides, have relatively large surface areas and may be rich in adsorped trace elements (of river, estuarine and marine origin). These particles are eventually incorporated into deep-sea sediments. Relative to these sediments the particulate material is deficient in Mn, Co, Ga, V and Ba, contains about the same amounts of Cu and Pb, and is enhanced in Zn. Continentally derived aeolian dust particles will also make a contribution to the total particulate material in the surface layers of seawater.

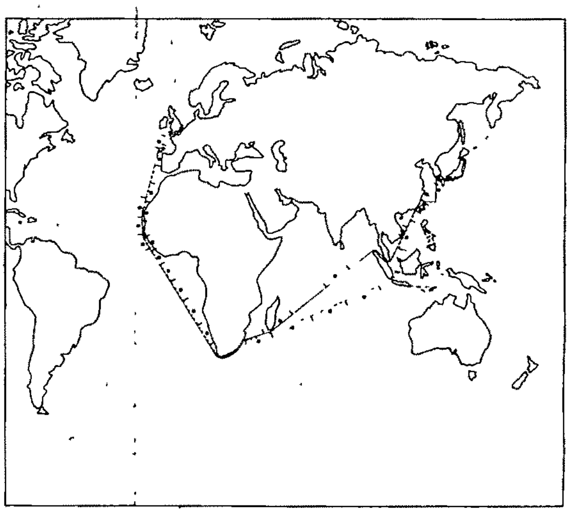

Fig. 1 Track of the MV Cyclops. Solid line, UK–Japan run; broken line, Japan–UK run. Particulate samples were collected over the intervals indicated by the lines perpendicular to the track; solid circles indicate the central positions of the sampling sites.

Particulate material in the upper layers of seawater undergoes considerable differentiation before and during its incorporation into marine sediments. This differentiation includes chemical changes which occur during its descent

Table 2 Trace element concentrations in surface particulate material, marine sediments and marine plankton (concentrations in p.p.m.)

	Mn	Cu	Co	Ga	V	Ba	Pb	Zn
Mean: particulate material; this work	529	109	13	5	63	126	58	220
Mean: surface water particulate material; Gulf of Maine[4]	540	480	—	—	—	—	—	360
Mean: near-shore sediments[5]	850	48	13	19	130	750	20	95
Mean: Atlantic deep-sea sediments[5]	4000	130	38	21	140	700	45	130
Range: marine plankton[1–3]	4.5–17	12–65	<1–15	—	—	6–100	4–219	39–1510

down the water column, at the sediment surface–seawater interface, and during burial. For example, the particulate material contains an average of ~30% organic matter[6], whereas deep-sea sediments usually have only ~1% of this component. Further, carbonate and siliceous shell debris are often lost from the sea floor by dissolution. It is not known whether or not the trace elements associated with these components are retained in the sediment residues. So there are inherent difficulties in comparing the trace element compositions of particulate material from surface waters with those of marine sediments. Nonetheless, it may be concluded that, in general, the total particulate material is enriched in Zn, and to a lesser extent Pb and Cu, relative to marine sediments.

There are considerable variations in the published concentrations of trace elements in marine plankton, and for this reason a range of values rather than an average is given for each element in Table 2. In spite of this difficulty, certain overall trends in the distribution of trace elements in the plankton can be evaluated from these figures; relative to marine sediments this component is considerably deficient in Mn, and is enriched in Pb and Zn. The two latter elements are also enriched in the particulate material.

So it seems that the trace elements in total particulate material from the surface layers of seawater have a number of sources: Mn, Co and V are probably mainly present in continentally derived material and authigenic precipitates; Pb and Zn are probably located largely in plankton; and Cu and Ba are partitioned between the two components.

We thank the Blue Funnel Line for permission to make collections from their ships, the Master, Officers and Crew of the MV Cyclops, A. J. Woodward for help in the collection of samples and the Natural Environment Research Council for financial support.

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Received February 24, accepted March 25, 1975.

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Charge relationships of phosphate sorption

IT is generally accepted that the mobility of phosphate in soil systems is largely governed by sorption-desorption reactions[1,2], but the mechanisms involved in these reactions have remained largely obscure. Studies[3–5] have shown that sorption of phosphate by soils can be described by at least two Langmuir equations over a solution phosphate concentration range of up to approximately 8×10^{2} µmol l^{-1}. Interpretation of these data has been complicated by the lack of an equilibrium condition, implicit in the Langmuir equation, and by the limited number of data points on the sorption isotherms. An alternative approach described here provides a reliable basis for the development of the sorption mechanisms.

Study of anion sorption by hydrous metal oxides, as developed by Hingston *et al.*[6–3] is based primarily on shifts in points of zero charge and on the "adsorption envelope". This approach has been questioned[9], and the extrapolation of these concepts, developed using pure components, to soils has not been tested adequately. It has been demonstrated[10] that measurement of the increase in negative charge that occurs as a result of phosphate sorption by soils, may be useful in evaluating the sorption mechanism.

Using experimental techniques described elsewhere[11], and a matrix solution of 10^{-1} M NaCl, we have found that phosphate sorption data, after transformation according to the Langmuir equation, for each of four contrasting New Zealand soils are described by three distinct linear relationships (denoted as regions I, II, and III). The sorption data represent an equilibrium condition estimated by a graphical approach[11], and cover in detail a solution phosphate concentration range of up to 8×10^{2} µmol l^{-1}. Interaction of regions was eliminated using a method of successive approximations. The Langmuir sorption constants, relating to sorption energy (k) and sorption maximum (b) for each region, are given in Table 1. The striking feature of the data is that the k values for each region were similar in spite of very wide differences in mineralogical components considered important in sorption of phosphate. The variation in k values

Fig. 1 Relationship between the amount of phosphate sorbed and the difference (ΔNa) between sodium uptake by the sorbent in the presence and absence of phosphate at the same level of sodium addition. Points A, B, and C refer to data in Table 2. *a*, Okaihau soil; *b*, Fe gel.

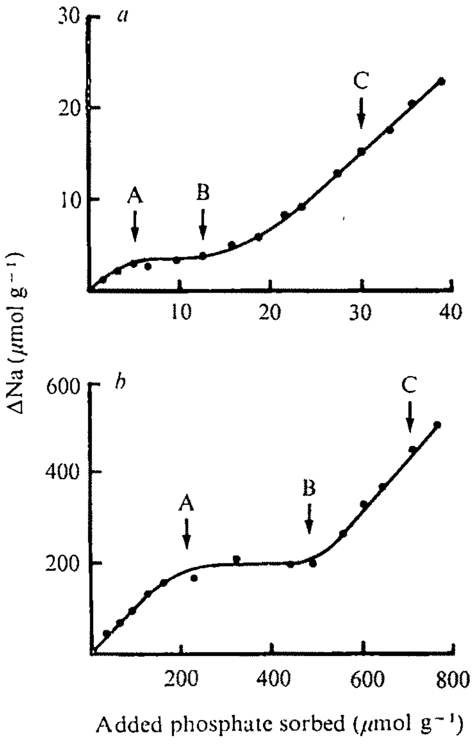

Table 1 Mineralogical components, *p*H, and Langmuir constants (*k* and *b*) for sorption of phosphate by four New Zealand soils

Soil	Mineralogical components	*p*H	k_I	k_{II}	k_{III}	b_I	b_{II}	b_{III}
				ml μmol^{-1}			μmol g^{-1}	
Egmont	Allophane	6.7	5,890	143	5.18	39.3	48.6	104
Okaihau	Kaolinite-gibbsite—secondary Fe_2O_3	5.1	7,380	208	5.02	21.4	33.3	55.9
Porirua	Micaceous vermiculite	4.6	3,280	145	2.51	4.19	9.19	17.1
Waikakahi	$CaCO_3$	8.1	2,970	158	8.90	1.40	2.61	10.5

Table 2 Sorption maxima for each of three regions of Okaihau soil and Fe gel (at 40 h in 10^{-4} M NaCl) and various data relating to Fig. 2

Sorbent	Region	*b* μmol^{-1} g	Saturation (%) at point A	B	C	ΔNa:phosphate*
Okaihau soil	I	4.19	93	100	100	0.76
	II	14.7	8	57	97	0
	III	22.4	<1	3	41	1.15
Fe gel	I	226	100	100	100	0.88
	II	242	5	100	100	0
	III	643	<1	5	39	0.96

*Average based on degree of saturation of regions I and III for various levels of overall phosphate sorption, calculated from respective *k* and *b* values.

between regions was approximately 20–50 times greater than that within a particular region. Each region corresponded to a well defined concentration range. For region I the concentration range was 0–0.7 μmol phosphate l^{-1}, for region II 1.5 to 25 μmol phosphate l^{-1}, and for region III 32 to >650 μmol phosphate l^{-1}. Within each concentration range at least 70 to 80% of the overall increase in sorption of phosphate was attributable to sorption in the corresponding region. Only the *b* values reflected the differences in the properties of the soils used.

These findings suggest that the origin of the deviation of phosphate sorption data from a single Langmuir equation arises from three distinct phosphate sorption mechanisms at sites which are common to each soil, rather than from phosphate sorption at different soil components, as suggested in other studies[3,5]. Although calcium carbonate is the dominant mineralogical component in Waikakahi soil, there is abundant evidence to suggest that this component is of minor importance in phosphate sorption[2], relative to the Fe and Al components present. The only possible sites for phosphate sorption common to all soils are those of the type M-OH (M = Fe or Al) which occur at the surfaces of hydrous metal oxides and short range order aluminosilicates, at the edge faces of crystalline aluminosilicates, and on Al (Fe) organic complexes. The *p*H dependence of charge at these sites[12,13], combined with that of the distribution of phosphate species in solution, suggest that the three regions of the phosphate sorption isotherm, each conforming to a Langmuir equation, may arise from three distinct phosphate sorption mechanisms at such sites.

This concept was tested by the change in negative charge during sorption of phosphate as measured by cation uptake. The difference (ΔNa) between the uptake of sodium by the sorbent during sorption of phosphate and that in controls containing the same initial sodium additions but no phosphate was determined. To facilitate the measurement of small changes in Na uptake in response to sorption of phosphate, a matrix solution of 10^{-4} M NaCl was used. Where applicable, phosphate was added as a solution of Na_2HPO_4 adjusted to *p*H 6.5. A 40-h sorption period was used. Sorption data obtained in this way are also represented by the same three distinct concentration ranges or regions, each conforming to a Langmuir equation. Values for *k* are comparable to those obtained for the equilibrium condition. This suggests that the non-equilibrium (40 h) condition is satisfactory within the scope of the experiment. Non-equilibrium *b* values for the Okaihau soil (Table 2), are however, considerably lower than equilibrium *b* values for the same soil (Table 1). This reflects the effect of the low ionic strength of 10^{-4} M NaCl on the rate at which equilibrium is attained[11].

Point A on the ΔNa phosphate sorbed curve (Fig. 1) for Okaihau soil and hydrous ferric oxide gel (Fe gel) corresponds approximately to the sorption maximum of region I. Point B corresponds to significant phosphate sorption in region III, and this is associated with an increase in ΔNa (Fig. 1, Table 2). Point C corresponds to continued sorption in region III.

Data in Fig. 1 and the ratio ΔNa:phosphate sorbed (Table 2), demonstrate that phosphate sorption in regions I and III involves an increase in the negative charge of the surface, whereas no change in surface charge occurs during sorption in region II (ΔNa is constant and ΔNa:phosphate = 0, based on sorption of phosphate in each region). The data also suggest that charge increases by approximately one equivalent per mole phosphate P sorbed in regions I and III. The somewhat lower value obtained for Okaihau soil in region I probably arises from the interference of cations, originally present in the soil, in charge balancing. This gives a lower uptake of added sodium at lower levels of phosphate sorption. Similar data have also been obtained for Egmont soil.

We suggest that the reaction mechanisms represented in equations (1), (2), and (3) below are responsible for sorption of phosphate in regions I, II, and III, respectively:

$$M-OH_2^{+}+H_2PO_4^{-} = M-H_2PO_4+H_2O \quad (1)$$
$$M-OH +H_2PO_4^{-} = M-H_2PO_4+OH^{-} \quad (2)$$
$$M-OH +HPO_4^{2-} = M-HPO_4^{-}+OH^{-} \quad (3)$$

The change in surface charge induced by each mechanism is in accord with changes in sodium uptake during phosphate sorption (Fig. 1, Table 2).

Additional evidence for these reaction mechanisms has been obtained from *p*H data and flocculation studies. During sorption of phosphate by Fe gel, *p*H increased rapidly beyond point A (Fig. 1, Table 2), corresponding to saturation of region I and significant sorption in region II (OH^- release; equation 2). Furthermore, a dramatic increase in the extent of flocculation of the Fe gel was also observed beyond point A (charge neutralisation; equation 1). Complete flocculation was maintained (zero charge increase; equation 2) until region III made a significant contribution to overall phosphate sorption (point B), when dispersion occurred (charge accumulation; equation 3).

Recent work in this laboratory (J. R. McLaughlin, personal communication) suggests that sorption of phosphate in regions I and II, corresponding to equations (1) and (2), results in the development of a "ferric phosphate-like" surface, and that sorption of phosphate in region III corresponds to sorption of phosphate as a potential determining ion at this surface. Such a mechanism would still result in unit increase in surface negative charge per mol phosphate sorbed in region III (Fig. 1, Table 2).

Although sorption of phosphate has previously been attributed to one or more of the mechanisms proposed above[6-10,14], this study represents the first quantitative evaluation of the relative importance of each mechanism over a wide solution phosphate concentration range. Our study extends the work recently reported by Rajan[12] in three distinct ways: first, sorption of phosphate by both soils and Fe gel has been studied in detail over a concentration range relevant to the soil solution; second, sorption has been studied at approximately the natural *p*H of the soil, covering the range of 4.6 to 8.1; and third, evidence for the mechanisms proposed has been obtained not only from charge and *p*H relationships, but also from an interpretation of sorption of phosphate based on the Langmuir equation.

Finally, our work also shows that a significant proportion of phosphate may be sorbed without increase in surface negative charge, a condition that has been implicit in the phosphate sorption mechanisms proposed by Hingston *et al.* [6-8].

One of us (J.C.R.) acknowledges a University Grants Committee Scholarship.

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Received February 12; accepted March 25, 1975.

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Liquid crystals as lamellar reservoirs reduce thinning by drainage

FOAMS and thin liquid films have provided important model systems for theories of water–surfactant interactions in colloidal systems[1,2]. Particular interest has been focused on the stability and structure of black films, since they represent a theoretically attractive system with implications for both physical and biological sciences. Until now drainage studies of thin films have concerned films formed from dilute surfactant solutions, which are of limited stability (that is, with a lifetime of a few minutes).

Foams formed from aqueous solutions, in which a lyotropic liquid crystalline phase is present in equilibrium with a micellar surfactant solution, are of noticeably greater stability than foams formed from micellar solutions alone[3,4]. Two mechanisms have been suggested for this. First, the liquid crystalline phase may concentrate in the plateau borders and thereby reduce film thinning by drainage. Second, the liquid crystalline phase may act as a reserve for the components which comprise the foam lamellae, so that drainage thinning or thinning under mechanical deformation of the foam lamellae, which would otherwise occur, is counteracted[3]. We provide here the first direct experimental evidence of the second of these mechanisms in the stabilisation of films. Further, we show that a lyotropic liquid crystalline phase introduced mechanically into a foam formed from a micellar solution, spreads through a foam by way of the lamellae, and concentrates in the plateau borders.

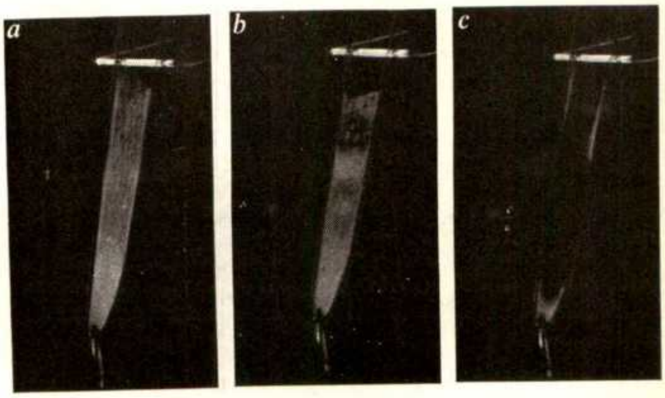


Fig. 1 Drainage of films formed from a micellar solution of 19% sodium caprylate, 7% decanol, 74% water, and stretched from 6 mm² to 100 mm². *a*, After 15 s; *b*, after 1 min. *c*, after 2 min. Film lifetime 150 ± 45 s. Film size 4 × 25 mm.

Films were drawn on a U-shaped glass frame (made from glass rod 0.5 mm in diameter) arranged so that the U opened upwards. The vertical arms of the U, separated by 4 mm, were closed by a horizontal glass barrier, which could be raised or lowered vertically to stretch the film.

The moveable horizontal glass barrier was set 4 mm above the base of the frame, and the frame was immersed in an aqueous solution. It was then removed, and any film that had formed (the area was 6 mm²) was immediately stretched, by moving the horizontal barrier out to an area of 100 mm². The lifetime of the film was measured, and drainage of the film was observed by watching the interference patterns produced by light incident at 45°. A micellar solution of 74% water, 19% sodium caprylate and 7% decanol was separated from a lamellar liquid crystalline phase of composition 38% water, 28% sodium caprylate and 34% decanol by the ultracentrifugation of a mixture of 70% water, 20% sodium caprylate and 10% decanol[5,6]. The micellar solution was used in the first film-forming experiments. The second series of film-forming experiments was carried out using the same micellar solution, but a 5 μl drop of the separated liquid crystalline phase was placed on the horizontal film barrier before immersion in the micellar solution.

The drainage of the film formed from the micellar solution alone is shown in Fig. 1*a*, *b* and *c* (after 15 s, 1 min and 2 min, respectively). The interference fringes initially present disappeared successively, and a black film formed from the top of the film downwards, extending to 5 mm below the upper barrier after 1 min, and becoming essentially complete after 2 min. A mean lifetime of 150 ± 45 s

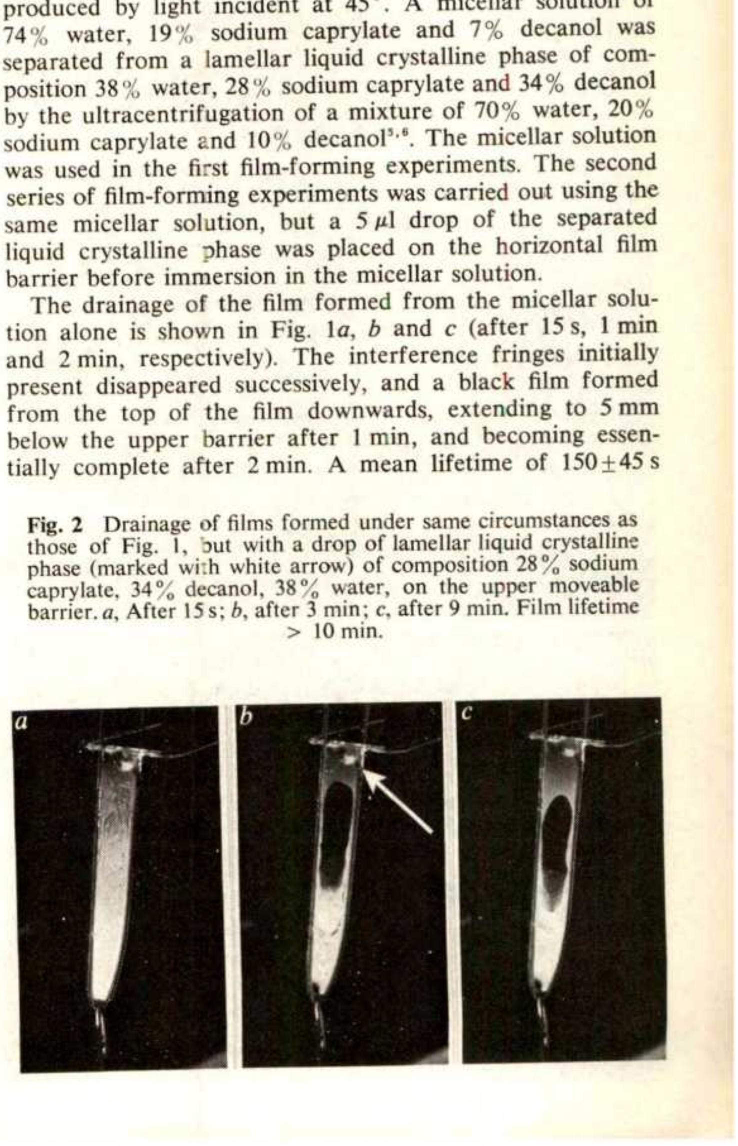
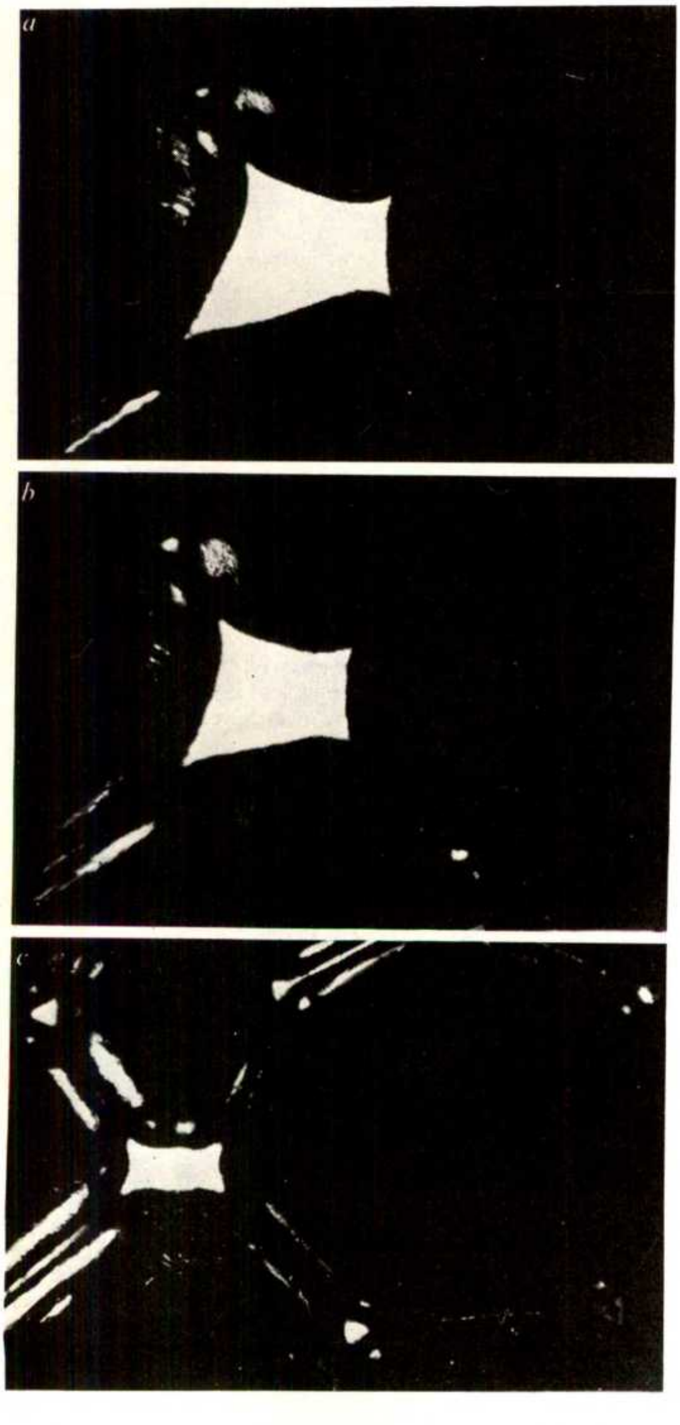


Fig. 2 Drainage of films formed under same circumstances as those of Fig. 1, but with a drop of lamellar liquid crystalline phase (marked with white arrow) of composition 28% sodium caprylate, 34% decanol, 38% water, on the upper moveable barrier. *a*, After 15 s; *b*, after 3 min; *c*, after 9 min. Film lifetime > 10 min.

(mean deviation 14 s) was observed for the film. The drainage behaviour observed when a liquid crystalline phase was present on the upper film barrier is shown in Fig. 2*a*, *b* and *c* (after 15 s 3 min and 9 min, respectively). After 3 min, a black film had formed from 4 to 15 mm below the upper barrier, and below the black film interference patterns were observed. A silver film existed both above the black film and around it in the immediate neighbourhood of the glass frame. After 9 min, the black film region had decreased in size, and a silver film formed from the top downwards. The lower region of the film showed interference patterns, indicating a multilayer structure. When a liquid crystalline phase was present, film thinning was prevented, as is indicated both by the persistence of the interference patterns in the lower half of the film and by the spreading of a silver film over the black film in the neighbourhood of the liquid crystalline phase. The lifetimes of films formed in the presence of the lamellar liquid crystalline phase exceeded 10 min. These data demonstrate that the presence of a liquid crystalline phase in contact with a drawn film increases the film stability.

Fig. 3 Microphotographs (×30) of a foam formed from the micellar solution of the composition of the film shown in Fig. 1, to which a liquid crystalline phase, of composition given in Fig. 2, has been added mechanically at time $t = 0$. *a*, $t = 10$ s; *b*, $t = 3$ min; *c*, $t = 17$ min.



A foam was formed from a micellar solution of the composition already described, and observed in a 1-cm-thick cuvette with a polarising microscope. The foam initially contained no liquid crystalline phase and was only weakly birefringent. A drop (5 μl) of the liquid crystalline phase separated as described already, was placed mechanically in the foam using a fine platinum wire, and the system was observed visually. Photographic records in polarised light were made over a period of 17 min. The results are shown in Figs 3*a*, *b* and *c* (after 10 s, 3 min and 17 min, respectively). The liquid crystalline phase appears initially as a large, light region, which gradually shrinks. This phase was observed to spread into the foam lamellae, which become thicker and show stronger birefringence, and to concentrate in the plateau borders, which were observed as triangles of light of gradually increasing intensity.

The observations of films demonstrate that the spreading of a liquid crystalline phase over a film, to prevent drainage thinning[1,2], provides one mechanism for the stabilisation of films. The observations of foam show that a liquid crystalline phase concentrates in plateau borders, and can thus be distributed throughout a foam.

We thank Professor S. Friberg for advice and discussion and the Swedish Board for Technical Development for financial support.

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Comparison of associations of birth order with intelligence test score and height

No accepted genetic model can explain a true association of any human attribute with birth order. This view was not understood when Galton reported on the overrepresentation of firstborns among men of eminence. Recently, scientists have been intrigued with the possibility that studies of birth order may aid understanding of the environmental sources of differences in temperament and intelligence. Many such studies have been handicapped by the confounding effects of family size, which have been difficult to eliminate in spite of the application of a variety of techniques.

This paper examines the consistency of the association of birth order with adult intelligence test scores on the one hand and with adult height on the other. Test scores and height are associated attributes[1] that have been presumed to reflect certain common antecedents of development. Both test scores and height also relate to family size[2]. Our comparison of the association of intelligence test score and of height with birth order, while keeping family size constant, will help to separate antecedents of intellectual development from those of somatic development.

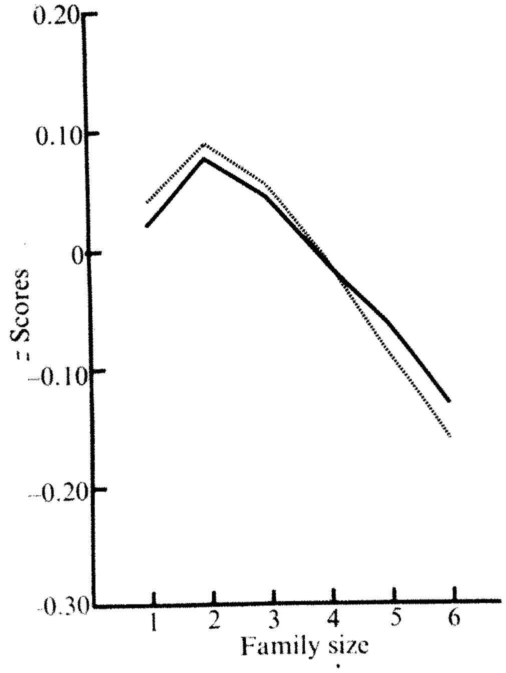
We have demonstrated[3] that birth order is related to intelligence test score and this relationship points to environmental effects, whether physical and biological, such as nutrition, or socio-psychological such as intra-familial socialisation. To distinguish between these two types of factors, we used height, which can be assumed to be related to physical and biological factors such as nutrition and childhood infections[4,5]. If height shows associations with birth order similar to those shown with intelligence test score, physical and biological hypotheses might best explain both. If, however, there is no association between birth order and height, then socio-psychological hypotheses may best explain the demonstrated variations of intelligence test score with birth order.

The data reported here consist of the coded records of the Dutch preinduction military examination for individuals born during 1944–46. These data are given in full elsewhere[6]. At 19, each young man is required to appear for an examination. The examination records were analysed with respect to class scores (1–6) on the Raven Progressive Matrices, information on birth order and family size, on father's occupation and on height.

The study population was incomplete in two respects. First, we excluded men from families containing more than seven children. Men who could show that three older brothers had served in the armed forces could claim exemption; by our estimates the loss of subjects by exemptions in families of less than seven was negligible. Second, some men were found incapable of taking the intelligence test (5.8%), and an additional 0.2% were not examined because they were either too short or too tall.

Figure 1 presents the contrast, in standardised normal deviates (z scores), for intelligence test score and height for all subjects grouped according to family size. The graph shows that family size is associated with both stature and test score—in general, individuals from smaller families are taller and brighter than those from larger families. Both height and test score in single-child families, however, are exceptions to this generalisation.

Fig. 1 Height (—) (cm) and intelligence (– – –) (Raven test score) by family size for the study population (n = 234,837).



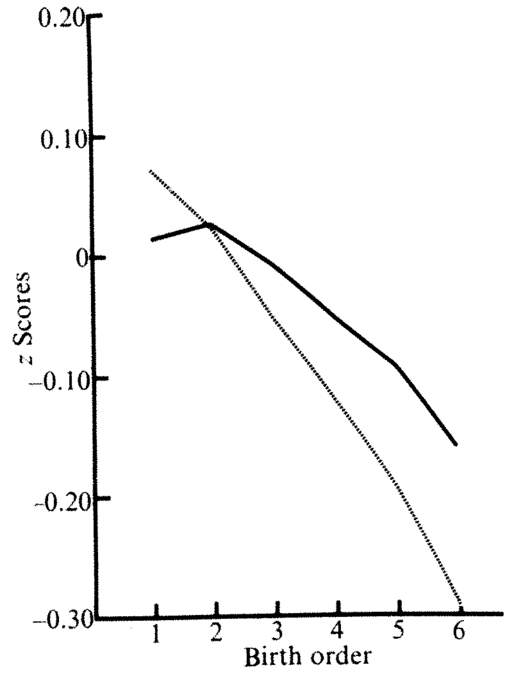


Fig. 2 Height (—) (cm) and intelligence (– – –) (Raven test score) by birth order for the study population (n = 234, 837).

Figure 2 contrasts the distributions for test score and height among all subjects by birth order. Intelligence test score and height present a configuration by birth order different from that for family size. The birth order gradient for intelligence test score shows the expected consistent decline from the first to the sixth birth rank. The gradient for height is less steep and the firstborn are shorter than the secondborn; a consistent decline in height for successive ranks begins only with the secondborn.

The z-score values for test score and height by birth order within each consecutive family size are shown in Fig. 3. Test scores show a consistent pattern for birth order, with the exception of only-child families. Within each family size, the firstborn always have higher test scores than the subsequent offspring. The regular declining gradient with successive birth orders is broken in only one instance, namely, the fourth child from five-child families. A stringent non-parametric test for discrepancy from ideal birth order[3] indicated that these results are statistically significant ($P < 0.001$).

The data for test scores also show a consistent pattern with regard to family size. For each birth order, members of small families have higher mean test scores than members of larger families, again excepting only-child families. There are two additional irregularities, at the second rank in three-child families and the fifth rank in six-child families. Family size effects on test scores are thus less consistent than birth order effects especially for birth rank five. Only earlier birth ranks meet the stringent requirements of our non-parametric test.

The values for height are quite dissimilar. There is no consistent relationship with birth order when family size is controlled. Actually, for two, three, and four-child families, there is a slight increase in height with increasing birth rank. In five and six-child families, the mean values are almost in a straight line. Thus, birth order effects with constant family size are not statistically significant. By contrast, height bears a consistent relationship with family size with constant birth order. With each successive increase in family size (except for single-child families) there is a consistent decline in mean height. The family size effects for height are statistically

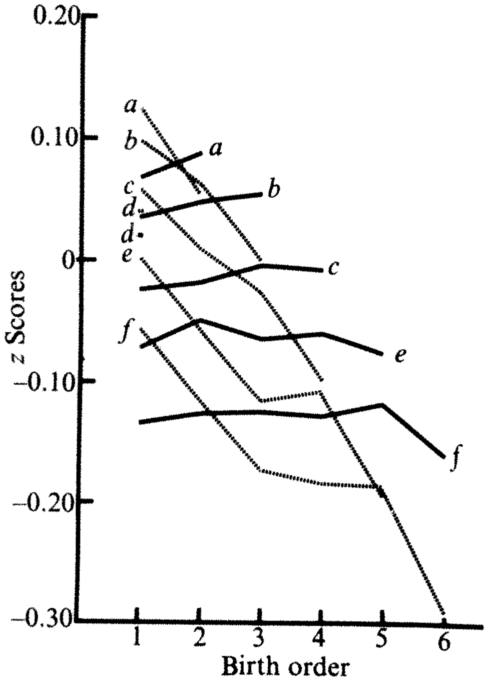


Fig. 3 Height (—) (cm) and intelligence (– – –) (Raven test score) by birth order within family size for the study population ($n = 234{,}837$). Family size: *a*, 2; *b*, 3; *c*, 4; *d*, 1; *e*, 5; *f*, 6.

significant ($P < 0.001$). Intelligence test score and height therefore diverge in relation to birth order.

Birth order and family size distributions for test scores and height were further analysed within social class. Social class was assigned according to father's occupation at the time of the son's military examination. Occupations were ordered into several occupational categories, but for convenience were finally reduced to only two classes: manual and non-manual. As expected, there were social class gradients for both test scores and height.

Social class does not explain either the test score or height gradients by birth order and family size. Among the manual social class the pattern for the total study population was replicated in all essentials for both test score and height. Among the non-manual class birth order effects for test score and for height also were similar to those for the total study population. Family size effects, however, were consistent for height but less consistent for test score: members of two, three, and four-child families scored higher than those of five and six-child families for given birth ranks, but within smaller sized families, family size effects were inconsistent and distinctly less marked.

The difference in the relationships of intelligence test score and height with family size and birth order indicates that the antecedents of the distributions of the two variables are not entirely shared. The association of test scores with birth order holds at virtually all levels of family size and social class, although the association with family size does not hold under all conditions. In contrast, the association of height with family size holds at all levels of birth order and social class, whereas the association with birth order disappears. The persisting birth order effects for test scores but not for height suggests that antecedents of adult intelligence are likely to stem from family environment and intrafamilial socialisation. Height is an index of the physical and biological environment during growth and its persistent relationship with family size is consistent with environmental effects. It seems, however, that the fullest development of intelligence requires more than nutritional adequacy and good physical health.

We thank the Department of Defence of the Netherlands for permission to use the preinduction examination data and acknowledge statistical consultation by Dr Janet Wittes, Assistant Professor, Hunter College, City University of New York. This work was supported by National Institutes of Health grants to L.B. and M.W.S.

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Received November 25, 1974; accepted March 10, 1975.

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Experimental playbacks show vocal mediation of intergroup avoidance in a forest monkey

DISTINCTIVE vocalisations, audible over long distances, have been reported among many forest primate species, ranging from prosimians to apes[1-5]. Such loud calls have often been hypothesised to mediate territorial or other forms of spacing between conspecific groups, and indeed audibility over distances sufficient to serve in intergroup communication is one of their defining characteristics. Nevertheless, evidence in support of such hypotheses is primarily indirect (but see ref. 1), and investigation of the responses of animals to these calls is generally lacking.

I report here the response of gray-cheeked mangabeys, *Cercocebus albigena*, to playback of recorded vocalisations, made in conjunction with an observational study of this species in the Kibale Forest Reserve, western Uganda, between March 1972 and April 1973. The study took place in evergreen rain forest near the Kanyawara Forest Station (0° 34′N, 30°22′E), where human influence on the forest was minimal and there was no hunting of primates[6,7]. Mangabeys were found in spatially extended but socially cohesive groups of between 6 and 28 individuals, using home ranges several km^2 in extent[6,7].

Vocalisations were recorded from recognisable individuals using a Nagra III tape recorder and a Sennheiser MKH 804 directional microphone. Study concentrated on two habituated groups within which all individuals were recognisable. Records of responses to naturally occurring calls were kept during monthly periods when the mangabeys were followed systematically; playback experiments occurred outside those periods. Playbacks required two observers in radio contact, one to follow the test group and the second to operate the playback equipment. On each experimental day all movements and calls of group members were first noted during a control period of at least 30 min. A test vocalisation was then played back, using a Uher 4000 Report-L tape recorder and Kudelski DH external speaker, at approximately its normal intensity. Recording of movements and vocalisations was continued for at least the next 30 min. Because of the rapidity with which mangabeys were expected to habituate to such simulated encounters with other groups, several precautions were taken: only one experiment was conducted on a given day, and at least 2 d elapsed between any pair of tests; all calls were played back at least 75 m, and as much as 500 m, from the nearest mangabey; and, in each experiment, only a single call was played back to the test group. Thus mangabeys never witnessed the equipment in operation and in most cases never saw it at all. In these conditions, they gave

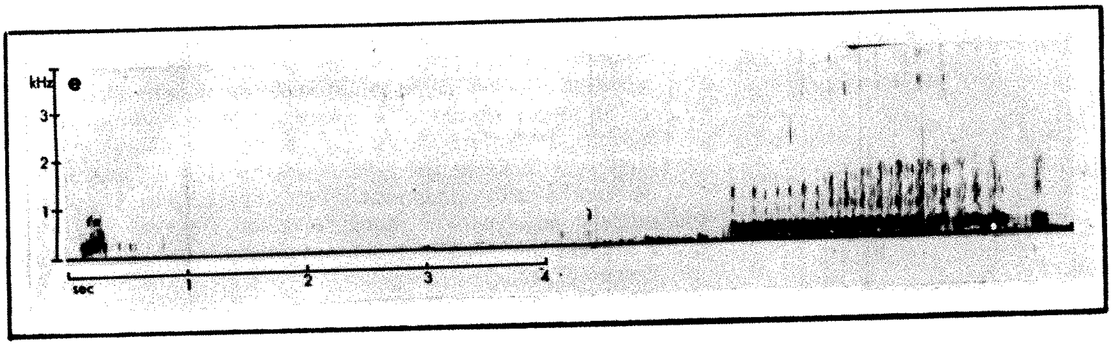


Fig. 1 Frequency-time display of whoopgobble call, analysed at double speed on Kay 6061B Sonagraph, wide band analysis; effective bandwidth, 150 Hz.

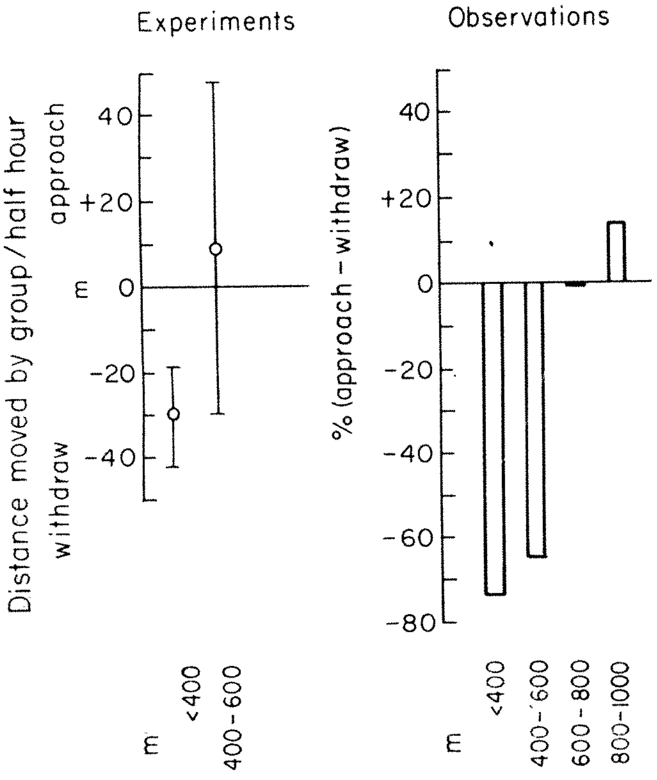
repeatable responses specific to both species and vocalisation.

The results reported here concern responses to the adult male vocalisations termed the 'whoopgobble' by Chalmers[8]. These calls consist of a low-frequency tonal whoop, then a 3–5 s pause, followed finally by a series of stacatto pulses comprising the gobble (Fig. 1). Because of their context and audibility, Chalmers suggested whoopgobbles 'might warn neighbouring groups of an occupied area'[8]. Playback experiments involved whoopgobbles recorded from known individuals in groups neighbouring or distant from the test group.

The response parameter most relevant to a possible intergroup spacing function is the net change in the movement of the groups as a whole after playback. Movements of the group centre of mass[6] during the 30 min preceding and succeeding the playback were compared and the group's net change in distance from the playback site was determined. The modal rate of group movement was 50–100 m per 30 min.

If a call is to maintain a minimal distance between groups, the response to playback of the call at less than this distance must be avoidance. More distant playbacks should be ignored or, conceivably, approached. Figure 2 compares the net changes in group movement in two series of experimental whoopgobble playbacks, one 'near' (100–200 m from the centre of the group, and more than 75 m from the nearest individual), the other 'distant' (400–600 m away). Changes in net group movement were consistent with the prediction. Near playbacks were never followed by group approach. Avoidance, on the other hand, was the exception in response to distant playbacks. The mean net displacement of the test group was away from near playbacks but slightly towards distant ones ($t = 2.68$, d.f. $= 13$, $P < 0.05$). Responses to naturally occurring whoopgobbles support the results of playback experiments. Whoopgobbles from a nearby group nearly always resulted in retreat within 30 min by the main study group, while those more than 600 m away did not (Fig. 2).

Fig. 2 Responses to whoopgobble calls near or distant from test group. Left, means and s. d. of net changes in group movement following playback; right, index of approach following naturally occurring calls. See text for further explanation. Sample sizes: (experiments) 7, 8; (observations) 19, 23, 7, 7, A slight but consistent difference in the distance from which playbacks and naturally occurring whoopgobbles were avoided may be due either to the playbacks possibly being quieter, and thus seemingly farther away, than naturally occurring calls; or to the fact that playback was from a height of about 2 m above ground, and was thus more highly attenuated than a call from the canopy.



To investigate the effect of the test group's location within its home range on its response to whoopgobbles, net changes in movement by the test group in its home range centre (defined as the central area which, during 1972–73, was occupied only by the test group) were compared with those in its home range periphery (the area which was occupied by other groups, as well as that test group). In an intergroup spacing system involving territorial defence, a spacing call heard nearby by the test group in its apparently exclusive area should be met with approach and (if the call is given by an intruding group rather than by playback equipment) displacement. On the other hand, if the call is heard near the extreme edge of the home range within an area of range overlap, withdrawal would be expected. Comparison of central and peripheral tests reveals no such trends. Net movement by the test group tended towards withdrawal in both cases and no significant differences appeared ($t = 0.47$, d.f. $=$ 13, $P \gg 0.05$). Again, responses to naturally occurring whoopgobbles parallel these results. When the main study group was in the centre of its own home range and the adjacent group trespassed deep into it, the main group still retreated after most whoopgobbles. The degree of retreat by the main group was not significantly greater ($P = 0.32$, one-tailed Fisher exact test) when the situation was reversed, with the main group trespassing near the heart of a neighbouring group's range.

Other responses which were both objectively measureable and clearly affected by playbacks included the spread of individuals within a group, the occurrence of immediate answering vocalisations, and the overall change in rate of whoopgobbles and other vocalisations by group members. Differences in these response measures, tests manipulating playback site rather than test group location, and observations of group movements and actual encounters all support the conclusion that response to whoopgobbles is a function of distance but not of position relative to the home range centre[6].

These results provide experimental support for the role of loud vocalisation in intergroup spacing, and indicate that playback techniques can be used successfully with free ranging primates. The unexpected finding that response does not vary with location indicates the more general value of playback techniques for investigating the pattern by which primate groups divide space among themselves, since the effects of such variables as identity

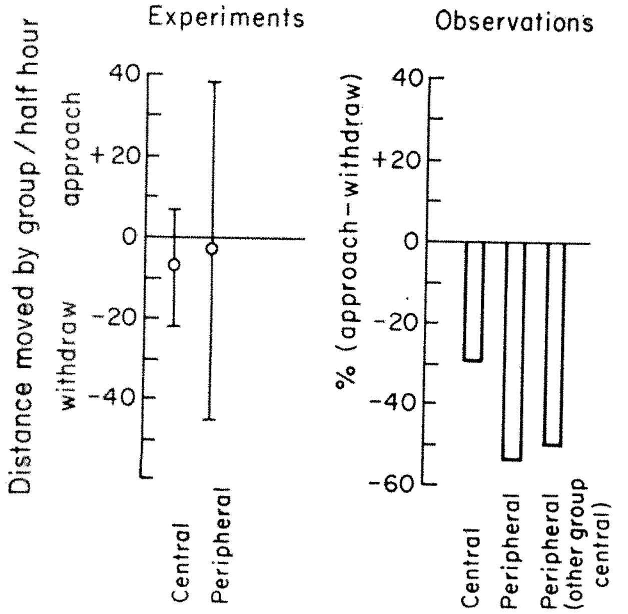


Fig. 3 Responses to whoopgobble calls when group is in the centre or periphery of its home range: comparison of experimental playbacks and naturally occurring calls. Conventions as in Fig. 2. Sample sizes: (experiments) 5, 10: (observations) 7, 30, 8.

of vocaliser, identity and size of vocalising groups, and level and location of resources can be investigated experimentally[6].

This work was supported by a grant from the National Institutes of Health. I thank M. S. Waser for assistance and T. Struhsaker and P. Marler for valuable discussion.

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Received February 6; revised March 12, 1975.

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Dynamic complexity in predator-prey models framed in difference equations

THE complicated dynamics associated with simple first-order, nonlinear difference equations have received considerable attention (refs 1–4 and R. M. May and G. F. Oster, unpublished). In an ecological context, equations of this type provide a powerful and realistic means of modelling the behaviour of animal populations with non-overlapping generations, typified by many arthropods in temperate regions. May[4] has shown that such models, incorporating density dependence, have three regimes of dynamic solution in their parameter space, namely (1) a stable equilibrium point; (2) bifurcating cycles of period 2^n, $0<n<\infty$, where n is a positive integer and (3) behaviour which has been termed chaotic, that is, cycles of any integral period or complete aperiodicity, depending on the initial conditions. May[4] has indicated that such complexity can also occur in the wider context of competition between two species, described by two first-order, nonlinear difference equations of similar form to those governing single-species growth.

In this paper, we illustrate the dynamics of a predator–prey model for populations with non-overlapping generations and show that the model yields patterns of behaviour closely analogous to those observed in the first-order (single-species) situation. These may be compared with results on the type of solution possible in predator–prey models framed as differential equations, which guarantee for a large class of models the existence of either stable equilibria or stable limit cycles[5].

The model we chose to investigate is an extension of the familiar Nicholson–Bailey host-parasite equations[6], which purport to describe the interactions between a population of herbivorous arthropods and their insect parasitoids. The original model is unstable for all parameter values[7]. Our extension, which eliminates this unrealistic behaviour, involves the inclusion of density-dependent self-regulation by the prey. The equations of the model are:

$$\begin{aligned} H_{t+1} &= H_t \exp[r(1 - H_t/K) - aP_t] \\ P_{t+1} &= \alpha H_t[1 - \exp(-aP_t)] \end{aligned} \qquad (1)$$

The self-regulation of the prey in the absence of predators has already been documented by May[4], with a stable equilibrium point for $0<r<2$, bifurcating cycles for $2<r<2.692$ and chaos for $2.692<r$. The choice of an identical form of density dependence in a predator–prey model therefore enables us to compare the parameter values at which chaotic behaviour ensues, and hence indicate whether the introduction of a predator makes the onset of chaotic behaviour more or less likely.

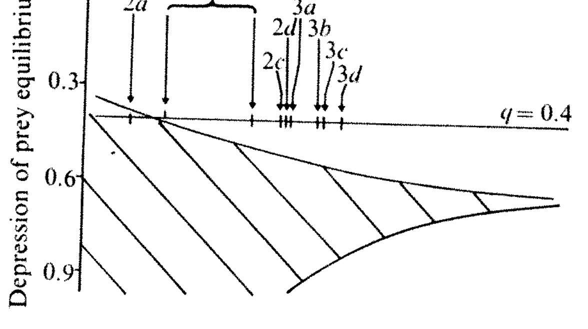
Analysis of the local stability properties of the model was performed using the method of Beddington[8]. The conditions for stability were found to depend on whether the roots of the equation

$$\begin{aligned} &\lambda^2 - \lambda(1 - r + \varphi) + (1 - rq)\varphi + r^2q(1 - q) = 0 \\ \text{where} \quad &\varphi = r(1 - q)/\{1 - \exp[-r(1 - q)]\} \end{aligned} \qquad (2)$$

lay within the unit circle. The parameter q is defined as the equilibrium prey population density, H^*, divided by the carrying capacity K; q is therefore a measure of the extent to which the predator can depress the prey below its carrying capacity. Application of the Schur-Cohn criteria[9] to equation (2) yields the demarcation of stable from unstable parameter space: this is illustrated in Fig. 1. Extensive numerical investigations within the domain of stable parameter space indicate that the equilibrium point is globally stable, although a Lyapunov function for the system has not been constructed.

Before considering the non-equilibrium behaviour of the model, we digress to point out the difficulties involved in recognising complicated dynamic behaviour in second-order models. *A priori*, we would expect limit cycles of integer period to be rare; it is more likely that the periods of any limit cycles that do exist will be predominantly non-integral, or indeed

Fig. 1 Stability boundaries for the predator–prey model, equation (1). The equilibrium point is stable inside the hatched area only. Simulations for the points marked on the transect $q = 0.4$ are illustrated in Figs 2 and 3.



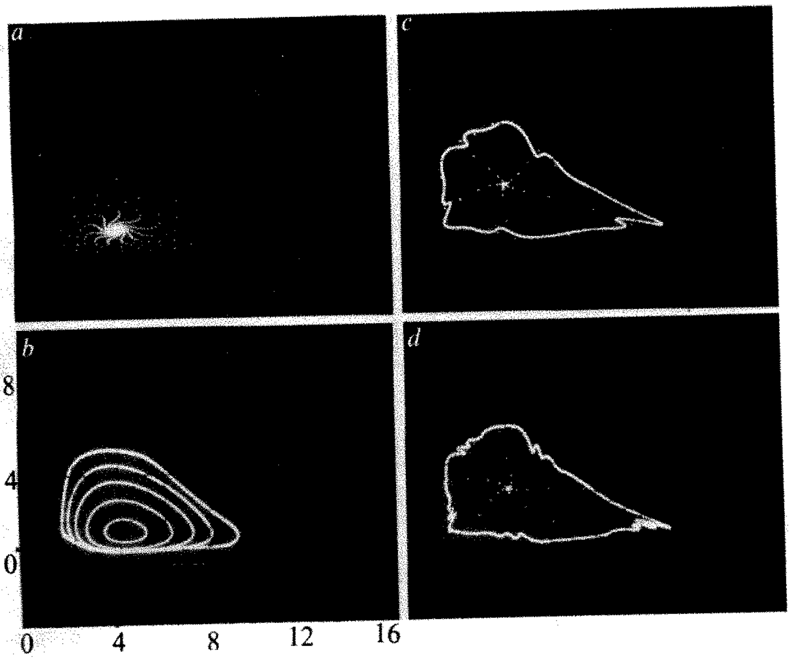


Fig. 2*a*, The realised trajectory for the model in the domain of oscillatorily stable parameter space $r = 0.5$, initial densities $H_0 = 8.6$, $P_0 = 1.1$. The plot indicates the time path followed to the equilibrium point. $q = 0.4$, $a = 1$, $K = 10$ throughout Figs 2 and 3. All plots are shown to the same scale. *b*, A succession of closed trajectories, realised after varying time periods from the initial point $H_0 = 3$, $P_0 = 3$. The parameter values, moving outward from the smallest curve, are (1) $r = 0.75$, (2) $r = 0.9$, (3) $r = 1.1$, (4) $r = 1.35$, (5) $r = 1.8$. Simulations indicated that the populations homed in on the closed curve trajectories from all regions of phase space. *c*, The realised trajectory of the model with $r = 2.1$ and initial densities close to equilibrium. The final closed curve is substantially different from the family of curves illustrated in *b*. *d*, as *c* with $r = 2.15$. The closed curve has now developed five kinked areas where the population points occur more frequently. Ordinates and abscissae are H_t and P_t respectively.

irrational. Correspondingly, limit cycles will be extremely difficult to detect simply by viewing the trajectory of one or both populations as a function of time. At most, this will permit a distinction only between integer cycles and other behaviour. In general, we would expect the existence of limit cycles in second-order models to be characterised by the populations following closed trajectories in phase space. We have performed our numerical simulations with this in mind: the non-equilibrium behaviour of the model was investigated by plotting the realised trajectory in phase space, using a storage oscilloscope on line to a computer.

The results of the simulations are presented in the context of a transect in parameter space (Fig. 1). The various types of behaviour of the model, corresponding to points on the transect, are illustrated in Figs 2 and 3. An initially stable point is succeeded by a hierarchy of stable limit cycles of increasing, non-integral period and increasing complexity, ultimately breaking down to cycles of integral period k (where $k = 5$), which then bifurcate to cycles of period $2k, 4k, \ldots, 2^n k$. These are followed by a regime of complex, but bounded, behaviour consisting either of limit cycles of high integral period ($2^n k > 10{,}000$) or of aperiodic chaos. The implications of the model in other zones of parameter space will be discussed elsewhere. The most important difference in these regions hinges on the basic period, k, of the integer cycles. Clearly, when compared with May's[4] results for a single-species model, the introduction of a predator has produced qualitatively new behaviour.

In the particular example illustrated predation has resulted in chaotic or high period limit cyclic behaviour for values of the prey growth rate parameter r only slightly below that in the single-species case. For other values of the parameter q however, we find that the onset of chaos occurs at values of r both significantly below (for example, $q = 0.30$, $r \approx 2.1$) and significantly above (for example, $q = 0.50$, $r \approx 3.3$) that of the single-species case ($r = 2.692$). A rough characterisation of what is obviously a somewhat complicated relationship is that the further the predator depresses the prey below its carrying capacity, the lower is the growth rate required for chaos. As a caveat, we note that the introduction of thresholds into population models (for example, an Allee[10] effect) will necessarily exclude the high amplitude limit cycles and larger chaotic domains. Nevertheless, our preliminary studies suggest that models incorporating such thresholds display effectively similar patterns of behaviour.

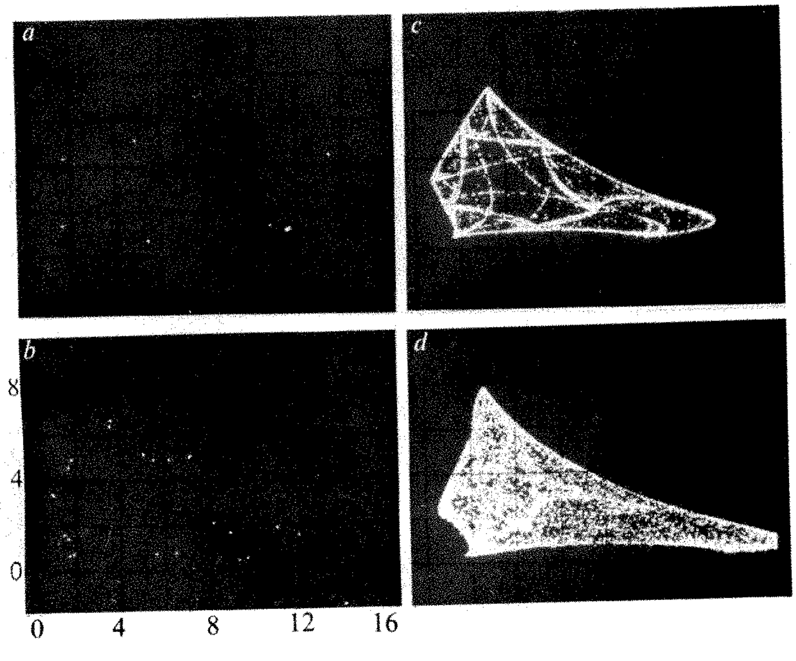


Fig. 3 *a* and *b*, Point limit cycles of period 5 and 20 realised after 100 iterations of the model from an initial point. Parameter values: (*a*) $r = 2.2$;(*b*) $r = 2.488$. Between these two points in parameter space lies a domain of stable ten-point cycles and for $r > 2.488$ cycles of period 40, 80 . . . have been found. *c* and *d*, Realisation of 10,000 points from the population trajectory started at initial densities $H_0 = 3$, $P_0 = 3$. *c*, $r = 2.55$. The structured, bounded figure shown possesses well defined areas where no points appear for iterations > 100,000. *d*, $r = 2.75$. Increased numbers of iterations yield a dense cover over the whole figure, within the limits of the oscilloscope resolution. Ordinates and abscissae are H_t and P_t respectively.

The existence of high period cycles or chaotic behaviour in predator-prey models (the distinction is unimportant for practical purposes) may be of considerable importance in interpreting the patterns of fluctuation shown by many arthropod populations in the field, as this implies the possibility of long term coexistence between predator and prey within well defined limits, but of a seemingly random nature. The temptation to ascribe such behaviour to 'environmental fluctuation' is obvious. Thus a recognition that such behaviour may occur in an extremely simple, entirely deterministic predator-prey model is of considerable importance.

We thank Professor Robert May for discussions.

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Prey death rates and rate of increase of arthropod predator populations

CENTRAL to our understanding of predator–prey dynamics is the relationship between the death rate imposed on the prey by the predators and the rate of increase, or numerical response, of the predator population. Most of the familiar mathematical models of predator–prey systems involve the assumption that there is a simple linear relationship between the number of prey killed and predator reproduction[1–4]. Although this assumption is valid for most insect host–parasitoid systems[2], the rate of increase of other predatory arthropods is a more complex function of the prey consumed. For successful reproduction, each instar must find and eat several prey to complete development. Thus the predator rate of increase will depend on the duration of, and the survival rate within each instar and the fecundity of the adults. Where models for the predator rate of increase have incorporated more complex nonlinear relationships[5,6], these relationships are of too abstract a character to allow simple experimental corroboration or refutation. In this paper we propose models that characterise the effect of prey consumption on the components of the predator rate of increase that are specifically designed to relate our hypotheses to field or laboratory data.

Much of the data available for testing these models comes from experiments in which a number of predators are exposed to a variety of prey densities. Thus it is necessary to utilise an expression for the number of prey attacked in terms of prey density N and predator density P. Indeed this is ultimately the form in which population models are likely to be framed. An instantaneous form for the number of prey attacked per predator, N_a, in time T assuming random search and random distribution of prey is, with unit area

$$N_a = aNT/[1+aT_hN+bT_w(P-1)] \qquad (1)$$

where a defines the rate of encounters between predators and prey, T_h is the handling time, b the encounter rate between predators and T_w the time wasted on an encounter between predators[7]. In many cases the assumptions of random search and random prey distribution are invalid[2,8] and in these situations a and b will be functions of the relative distribution of predator and prey.

For simplicity, and because this is the most usual type of experimental design, we explore the relationship between the prey death rate defined by equation (1) and the predator rate of increase for a single predator confronting a variety of prey densities. This simplifies equation (1) to

$$N_a = aNT/(1+aT_hN) \qquad (2)$$

the well known 'disc equation' of Holling[9,10].

Energetic considerations demand that a predator must allocate at least some food for maintenance. Accordingly this determines a threshold below which growth will not take place and eggs will not be produced. The growth rate of a predator g and the fecundity F will therefore be related to the energy intake I from food consumption by essentially similar models; thus

$$g = \delta(I-c) \qquad (3)$$

$$F = \lambda(I-c) \qquad (4)$$

where c is a constant determined by the maintenance energy

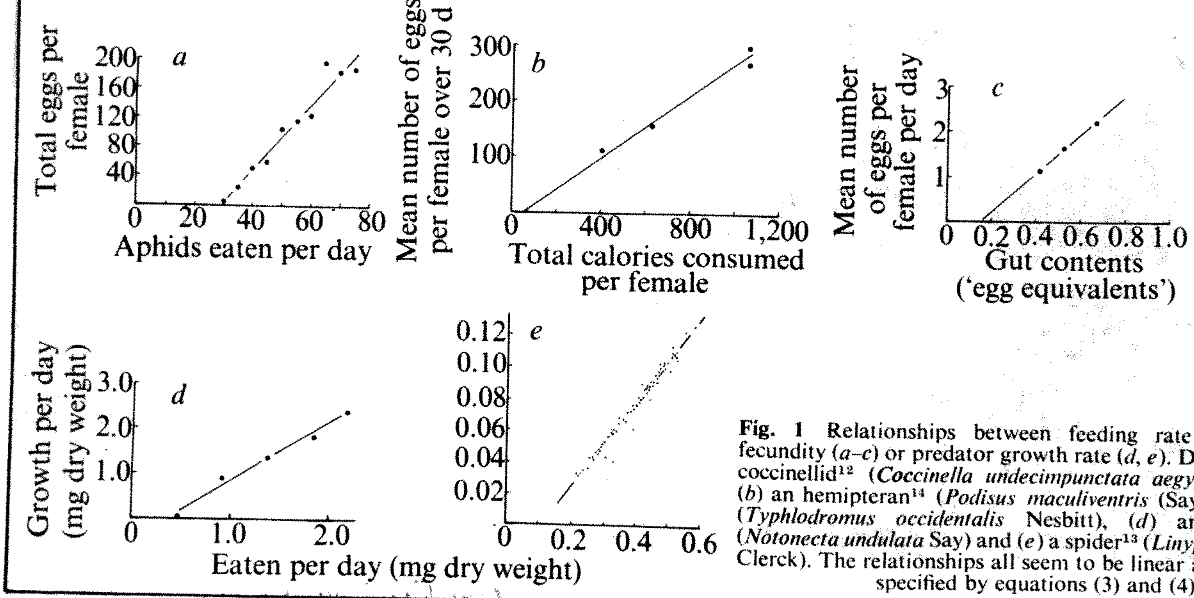


Fig. 1 Relationships between feeding rate and predator fecundity (*a*–*c*) or predator growth rate (*d*, *e*). Data are for (*a*) a coccinellid[12] (*Coccinella undecimpunctata aegyptiaca* Reiche), (*b*) an hemipteran[14] (*Podisus maculiventris* (Say)), (*c*) a mite[16] (*Typhlodromus occidentalis* Nesbitt), (*d*) an hemipteran[17] (*Notonecta undulata* Say) and (*e*) a spider[13] (*Linyphia triangularis* Clerck). The relationships all seem to be linear and of the form specified by equations (3) and (4).

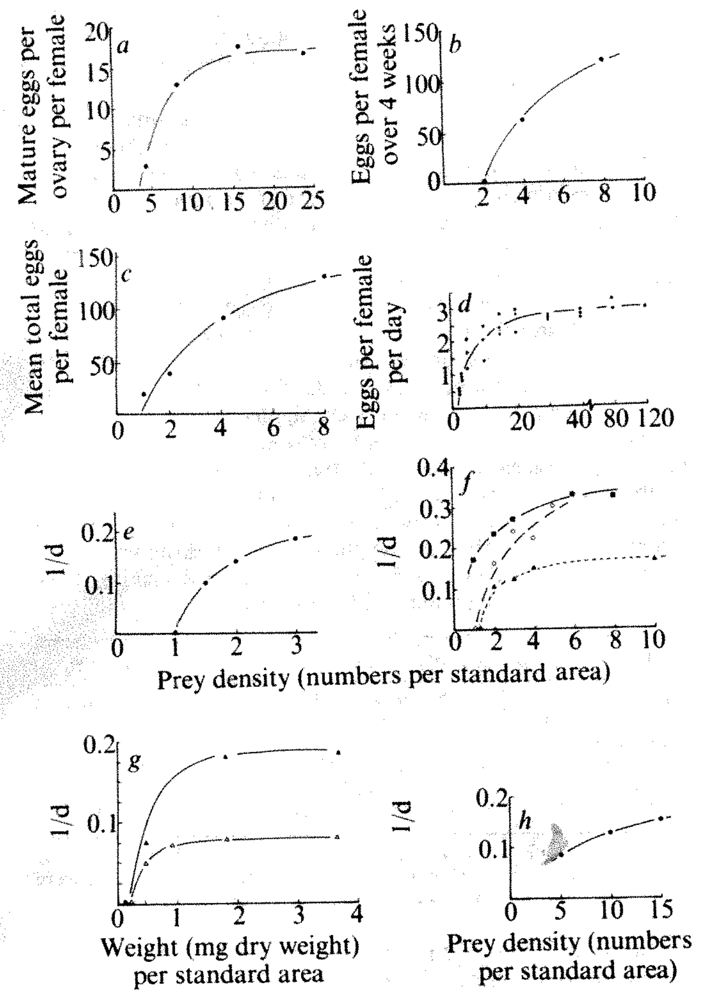


Fig. 2 Relationships between prey density and predator fecundity (*a–d*) or development rate (*e–h*). Data are for coccinellids (*a*, *e*) *Adalia decempuntata* (L.)[18] and (*f*) *Adalia bipunctata* (L.)[19]; hemiptera (*b*, *g*) *Notonecta undulata*[17] and (*c*) *Anthocoris confusus* Reuter[20]; and mites (*d*) *Phytoseuilus persimilis* Athias-Henriot[21] and (*h*) *Melichares dentriticus* (Berl.)[22]. All the relationships take the form of a negatively accelerating rise to a plateau, as predicted by equations (8) and (9), with positive intercepts on the prey-density axis. ■, Instar I; ○, instar II; ▲, instar IV; △, instar V.

requirements of the instar and the proportion of ingested food assimilated and δ and λ are appropriate proportionately constants (Fig. 1). The duration of an instar d can now be simply related to the growth rate by the observation that if W is the necessary weight gain within the instar, then the ratio W/g defines d, and the inverse of d the development rate. From equation (3) it follows that

$$1/d = (\delta/W)\,(I-c) \tag{5}$$

One important complication occurs when predators with a restricted food supply moult to the next instar at lower body weights than individuals with an abundant food supply[11,12], in extreme cases with a body weight below that at which they entered the current instar[13]. Nevertheless, the data available for organisms which exhibit this phenomenon can still be described by an equation of the same essential structure as equation (5)[13,14]

$$1/d = \alpha(I-\beta) \tag{6}$$

In this case however the constants α and β have no simple biological interpretation.

It now remains for us to relate both development rate and fecundity to prey density. Clearly the rate of ingestion I will be approximately proportional to the number of prey eaten. Thus as an approximation

$$I = kN_a \tag{7}$$

where k is the appropriate proportionately constant determined by the biomass of prey and the fraction of each prey killed that is utilised. We now use our simplified expression (2) to arrive at relationships for a single predator between fecundity and prey density and instar duration and prey density:

$$F = \lambda[kaN/(1+aT_hN)-c] \tag{8}$$

and

$$1/d = \alpha[kaN/(1+aT_hN)-\beta] \tag{9}$$

These relationships are supported by a variety of data (Fig. 2).

Ignoring mortality from other causes, if the frequency with which members of a population of predators die is normally distributed about a mean ingestion level μ_I with standard deviation σ_I (ref. 15), the proportion of the population surviving, s, to complete development within any particular instar at an ingestion rate I will be given by

$$s = 1/2\pi\int_{-\infty}^{z}\exp(-z^2/2)\mathrm{d}z \tag{10}$$

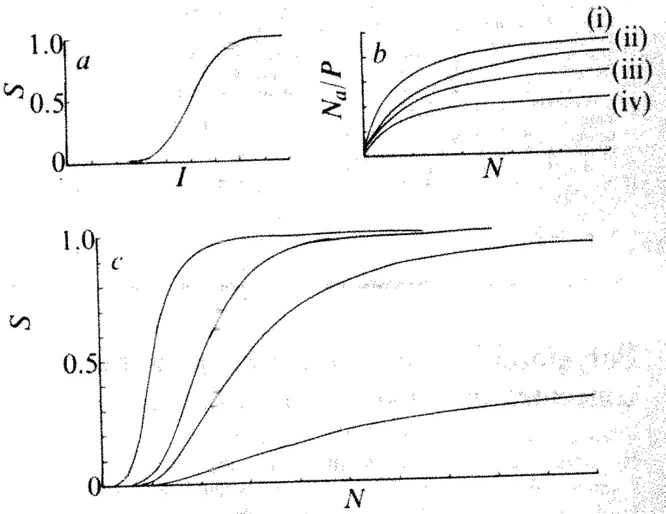
and

$$z = (I-\mu_I)/\sigma_I \tag{11}$$

It is now possible to utilise equations (2) and (7) and express s in terms of prey density N. Some possible relationships generated by this derived expression are illustrated in Fig. 3, which can be compared with the experimental data in Fig. 4.

Clearly any model which incorporates the same essential features as our equations (a threshold, and a saturation at high prey density) is likely to imply qualitatively similar relationships to those illustrated in Figs 2–4. Our aim has been, however, to develop models the parameters of which may be estimated experimentally and in which the underlying biological mechanisms are made explicit.

Fig. 3 Hypothetical relationships between (*a*) the proportion of individual predators surviving to the end of an instar and their mean feeding rates during that instar, and (*b*) predator feeding rates and prey density; the former is defined by equation (10), the latter by equation (2), for various values of a and T_h. The asymptotic rates of feeding defined by curves (i) and (ii) in (*b*) are just sufficient to lead to maximal survival in (*a*). Combining curve (*a*) with the curves shown in (*b*) produces (*c*), which relates survival to prey density. The hypothetical relationships in (*c*) should be compared with the experimentally derived curves shown in Fig. 4.



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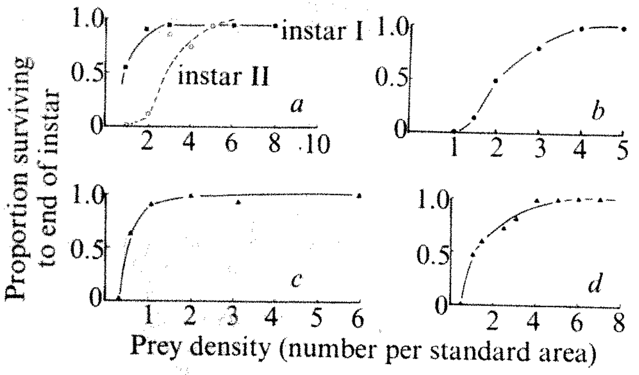

Fig. 4 Relationships between mean prey density during an instar and the proportion of individual predators surviving to the end of that instar. Data are for (*a*, *b*) coccinellids[18,23] (*Adalia decempunctata*, and *Adalia bipunctata*), (*c*) an hemipteran[15] (*Blepharidopterus angulatus* (Fall.) and (*d*) a spider[13] (*Linyphia triangularis*). ■, Instar I; ○, instar II.

Having documented well-corroborated relationships between the components of the predator rate of increase and the prey death rate, the task remains to investigate their implications for the behaviour of predator-prey systems. These problems, together with a more detailed treatment of the material in this paper, will be discussed elsewhere.

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Received February 10; accepted March 15, 1975.

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Polymorphism of erythrocyte potassium concentration in seaweed-eating sheep

INDIVIDUAL normal adult sheep have either a high or low erythrocyte potassium concentration, determined genetically by a simple allelic pair in which the gene for low K is dominant. High potassium (HK) animals have low erythrocyte sodium and low potassium (LK) animals high. Both phenotypes are present, and in all proportions, in 115 of 129 populations listed[1].

It is tempting to expect that such a striking physiological distinction would have a clear adaptive significance in an environment that offered an appropriate selection pressure. North Ronaldsay seems to be such an environment. The primitive native sheep of this small island in Orkney have been confined by a wall to the sea-shore for at least 140 years. Their diet is almost entirely of fresh seaweed and it is presumed that their intake of sea-salt is unavoidably high. These sheep number approximately 4,500 (ref. 2).

Blood samples were taken on the island from 113 adult sheep; 5 were HK and the rest LK. The average plasma sodium and potassium concentrations of the LK sheep were found to be about the same as those of LK mainland sheep, but the estimates for erythrocyte concentrations were significantly lower.

The diet of the Orkney sheep therefore does not seem to have affected plasma electrolyte levels, but the reduced values for the erythrocytes implies that the factors maintaining the electrolyte gradient across the erythrocyte membrane differ from those in mainland sheep. Although one phenotype is thus shown to have been modified, the exigencies of life on North Ronaldsay have not overwhelmingly favoured either phenotype.

Table 1 Blood electrolyte estimates for LK sheep, (mEq l^{-1})

Origin	North Ronaldsay	Scottish mainland
Number of sheep	108	314
Plasma Na	133.5 ± (0.51)	136.3 ± (0.32)
Plasma K	5.0 ± (0.05)	5.1 ± (0.04)
Erythrocyte Na	59.7 ± (1.44)	70.9 ± (0.94)
Erythrocyte K	9.8 ± (0.26)	19.9 ± (0.27)

Arithmetic means (s. e.) for North Ronaldsay sheep. Adjusted means ± s. e. for Scottish mainland sheep. (Values for the latter were calculated from the data of reference[3] by Mr E. A. Hunter.)

Our conclusion is that if there were any ecological significance in the polymorphism of blood electrolyte concentrations it remains obscure. A detailed account will be given elsewhere.

The authors acknowledge financial assistance from Trinity College, Cambridge and from the ARC. We thank the people of North Ronaldsay, in particular Mr R. J. Swanney and the North Ronaldsay Sheep Court, without whose enthusiasm and hospitality this study could not have been undertaken.

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Received February 10; accepted March 7, 1975.

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Antibody-induced cell-mediated damage to human endothelial cells *in vitro*

THE blood vessels of allografted organs are important sites for immunological injury[1–3]. *In vivo*, this is thought to be the result of interactions between antigenic determinants on donor vessel endothelium and recipient immunocompetent cells and antibodies. *In vitro*, damage to cultured pig endothelium by cytotoxic xeno- and alloantisera in the presence of rabbit complement has previously been described[4]. In addition, both canine[5] and human[6] endothelial cells are capable of stimulating allogeneic lymphocytes to blastogenesis and DNA synthesis, thus demonstrating the ability of endothelium to initiate directly an immunoproliferative response *in vitro*.

Antibody-induced cell-mediated cytotoxicity (AICC) has been reported previously, using a variety of allo- or xenogeneic target cells[7–9] and could be an important *in vivo* mechanism in the rejection of allografts. Here we describe damage to ^{51}Cr-labelled dissociated human endothelial cells *in vitro* by non-immune allogeneic lymphoid cells in the presence of anti-HL-A antibodies.

Endothelial cells were isolated from the vein of human umbilical cords by collagenase digestion, using a modification of the method described by Jaffe *et al.*[10–11]. Those cells were identified as endothelial in nature by light and electron microscopy, by their possession of typical inclusion bodies, by their typical growth pattern in culture and by their ability to stimulate allogeneic lymphocytes (for details see ref. 6). Neonatal lymphocytes (autologous to the endothelial cells) were separated from heparinised cord blood by Ficoll–Isopaque flotation. A small portion of the lymphocytes, approximately 0.1×10^6 cells was used to type the endothelial cell donor for HL-A, using the standard micro-lymphocytotoxicity test[12]. Both endothelial cells and lymphocytes were labelled with ^{51}Cr sodium chromate (Na_2CrO_4, Institutt for Atomic Energy, Kjeller, Norway) by incubating approximately 0.5×10^6 cells in 1 ml of culture medium containing 200–300 µCi ^{51}Cr for 90 min. The endothelial cells were usually more heavily labelled than the lymphocytes. The labelled cells were washed and filtered through sterile gauze before resuspension.

The anti-HL-A antisera used in each test were obtained from the battery of typing sera used in this laboratory for routine HL-A typing. The particular antiserum selected for each experiment had a specificity for at least one of the HL-A antigens possessed by the endothelial cell donor. The antisera were inactivated by heat (56 °C for 30 min) and diluted in foetal bovine serum (FBS). The basis for the selection of effector cell donors from previously non-alloimmunised individuals was a non-reaction with the particular HL-A antiserum used in each test. Peripheral blood lymphocytes were separated from these donors by Ficoll–Isopaque flotation. The AICC test is described in the legend to Fig. 1 (see also ref. 13).

The influence of the concentration of anti-HL-A antisera on the isotope release (endothelial cell cytolysis) is shown in Fig. 1. Release of ^{51}Cr from lymphoid target cells separated from cord blood of the endothelial cell donor is also shown for comparison. Incubating the labelled endothelial cells, either with heat-inactivated antiserum in the absence of effector cells, or with effector cells in FBS, always resulted in a lower isotope release than that observed by incubating the cells alone in FBS. In the presence of both antiserum and effector cells, a significant release of isotope was obtained for endothelial as well as lymphoid target cells in all five of the combinations tested. This was the case even at an effector–target cell ratio of 10:1. Increasing the number of effector cells fivefold, resulted in a relatively small increase in isotope release (approximately 25%). One of the antisera tested (anti-HL-A12) gave an isotope release of endothelial cell targets of approximately 35% at a dilution of 1:1,000. At this dilution, the percentage release for lymphoid cell targets was only 9%.

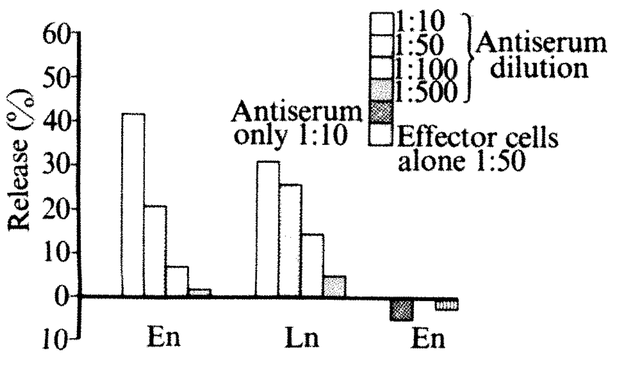

Fig. 1 2×10^3 ^{51}Cr labelled endothelial cells (En) or lymphocytes (Ln) were incubated for 18 h in the presence of 1×10^5 peripheral blood lymphocytes and various concentrations of an anti-HL-A3 antiserum diluted in FBS. All experiments were carried out in triplicate in the wells of round-bottomed microtitre plates in a total culture volume of 0.2 ml. Assay for ^{51}Cr release was carried out by transferring 0.1 ml of the cell supernatant to counting vials containing 4 ml of scintillation fluid (Insta-Gel) and counting in a liquid scintillation counter. The percentage release was calculated using the median of each triplicate culture from the expression:

$$\frac{\text{Experimental c.p.m.} - \text{Spontaneous c.p.m. in FBS}}{\text{Max. c.p.m. in Cetavelon} - \text{Spontaneous c.p.m. in FBS}} \times 100$$

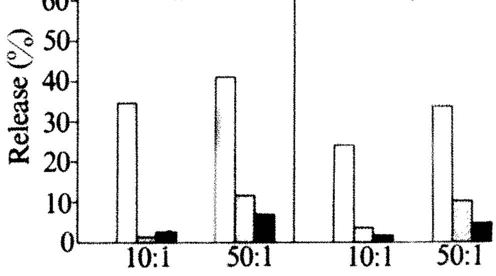

Fig. 2 The percentage release in AICC for labelled En (*a*) or Ln (*b*) target cells (□) in the presence of five times as many unlabelled En (////) or Ln (■) bystander cells. Effector–target ratio 10:1 or 50:1 as shown. Antisera (anti-HL-A12) dilution; 1:100. En and Ln target or bystander cells were obtained from the same donor. The experimental procedure was otherwise as explained for Fig. 1.

To determine if the cytolytic mechanism towards endothelial cells was similar to that operating towards lymphoid target cells in AICC, we investigated the effects of adding an excess of unlabelled target-autologous bystander lymphoid or endothelial cells to the targets under test. The results are shown in Fig. 2. At an effector–target ratio of 10:1, the addition of either five times as many unlabelled bystander endothelial or lymphoid cells to the labelled endothelial target cells clearly inhibited the release of isotope. Increasing the ratio to 50:1, in the presence of bystander cells, increased the lysis of target cells (compared with that for a ratio of 10:1), but did not completely compensate for the presence of unlabelled cells. In these experiments, increasing the number of effector cells always resulted in an increased isotope release. Therefore, the inhibition observed when unlabelled target-autologous bystander cells were added to the cultures, could not be caused primarily by an increased number of cells *per se*. Similar results were obtained in experiments using labelled lymphoid cells as target cells (Fig. 2*b*), where either adding unlabelled lymphocytes or endothelial cells greatly reduced the ^{51}Cr release otherwise observed.

These results indicate that human endothelial cells can be damaged by non-immune peripheral blood lymphocytes in the presence of anti-HL-A antibodies, even at rather low antibody concentrations. Complement-dependent cytotoxicity towards dissociated endothelial cells of both dog and pig, in the presence of cytotoxic allo- or xenoantiserum, has been reported previously[4,14]. In these experiments, high antibody concentration, as well as the presence of xenogeneic complement, was necessary to demonstrate cytolysis of the endothelial cells. All of the antisera used in our experiments were heat inactivated, and therefore the effects observed were by necessity complement independent.

Previous studies have indicated that the target structures on lymphoid cells in AICC are most likely the serologically defined HL-A antigens[9,13,15], although other interpretations cannot be ruled out. Our results imply that both endothelial and lymphoid target cells compete for the same cytotoxic effector mechanism (competitive inhibition, Fig. 2) in AICC. This suggests an apparent similarity or identity between the target structures on endothelium and lymphocytes; by inference possibly the HL-A antigens found on all human cell types tested so far[12] including endothelium[16]. On the other hand, specific cytotoxic xenoantisera for canine endothelium have been reported[17] suggesting the existence of antigenic sites on endothelium not found on lymphocytes from the same donor. Similar differentiation antigens on human endothelial cells and the possibility that these specific structures can also serve as targets in AICC, are now under investigation.

This work was supported by a grant from the Norwegian Research Council for Science and the Humanities.

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Received February 24; accepted March 13, 1975.

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T & B lymphocytes in thymus of SJL/J mice

The thymus reaches relative maximal development early in life followed by apparent programmed involution, which is eventually expressed in reduced functions of the T cell system. In the SJL/J mice we observed an interesting deviation of the normal thymus pattern, namely a secondary increase in thymus weight from the age of 7–9 months onwards (average weight of 2-month-old thymus being 60 mg compared with 130 mg in a 14-month-old normal mouse). The timing of this secondary thymus weight increase seems to coincide with the comparatively early, age-related, progressive decrease in the immuneresponsiveness of SJL/J mice described recently[1]. We therefore investigated the thymus population pattern in SJL/J mice in relation to age increase, using membranal antigenic markers as well as functional criteria. Based on antigenic properties of the cell surface of mouse thymocytes, the thymus population can be divided into two defined subpopulations. The major population (about 85%), which resides mainly in the cortex, possesses high levels of θ antigen and low levels of surface H-2 alloantigens. The remaining minor population has antigenic properties similar to peripheral T cells, namely low levels of θ and high levels of H-2 (refs 2 and 3). The immunological reactivity is usually attributed to the small, high H-2 subpopulation[4]. Thymocytes belonging to the B cell compartment are rarely found[5].

In this study, the presence of θ antigen and high levels of H-2 alloantigen on thymocytes was determined using a cytotoxic test. From the age of 4 months onwards a decrease in the percentage of θ-bearing cells could be detected in the thymus of SJL/J mice. The decrease was progressive with increasing age, namely 85% at the age of 1–2 months to about 50% at the age of 8–9 months and as low as 23% at the age of 15 months (Table 1). The NZB strain was the only other strain of mice reported to have an age-dependent decrease in the percentage of θ-bearing cells in the thymus[6]. A progressive age-related increase in the percentage of thymocytes carrying high levels of the H-2 alloantigen was observed (Table 1). In young SJL/J mice about 12% of the thymocytes carried high levels of H-2 antigen whereas at the age of 11 months the incidence increased to 58%.

Increasing incidence of immunoglobulin-bearing cells could be detected in SJL/J thymuses from the age of 5 months (Table 1), reaching a 50% incidence of IgG and 22% IgM-bearing cells in the thymus of 15-month-old SJL/J mice. The figures for IgG were higher than those of IgM. The possibility that thymocytes might carry both θ antigen and immunoglobulin determinants on their surface membrane was excluded by double labelling evaluation (see Table 1).

To check whether the surface immunoglobulins, as demonstrated by immunofluorescence, were synthesised by the cells themselves, thymus cells from 15-month-old SJL/J mice were treated with proteolytic enzymes and then kept in culture for several hours (as a positive control spleen cells were treated similarly). Surface immunoglobulins were evaluated using the direct immunofluorescence method[7]. Samples for immunoglobulin determinations were tested before and immediately after enzyme treatment and at 2 and 4 h after incubation. The capacity of the enzyme-treated cells to resynthesise immunoglobulins is shown in Fig. 1. The results indicated that the B cells were sensitive to trypsin or papain since a drop in surface immunoglobulins was observed following enzymatic treatment and recovery to normal levels was indicated 4 h after appropriate cultivation. Further analysis of these B cells present in the thymus of SJL/J mice was carried out by testing their functional ability to form anti-SRBC antibodies. Mice (10–12-month-old)

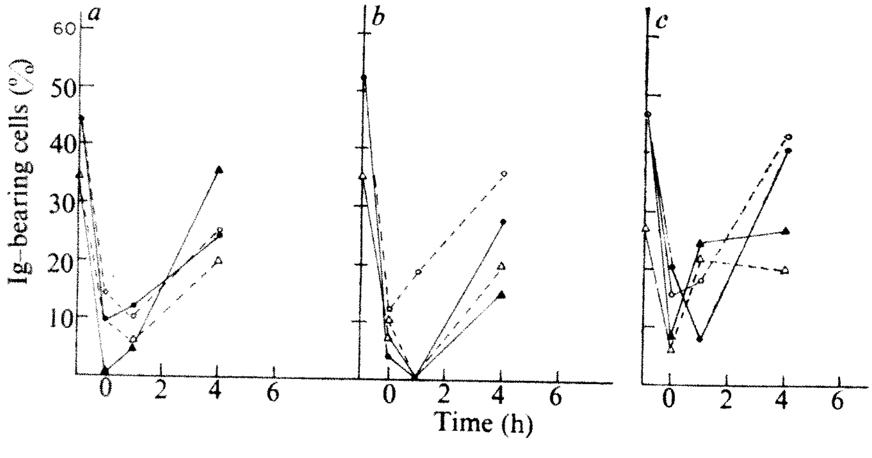

Fig. 1 Immunoglobulin production by SJL/J thymus cells after treatment with proteolytic enzymes. Thymus cells from 15-month-old SJL/J mice (*a* and *b*) or spleen cells from 2-month-old (*c*) animals were treated for 30 min (37 °C) with either (continuous line) trypsin (0.25% in Puck's solution) or (dotted line) papain (0.05% activated by 0.02 M dithiothreitol in the presence of 0.002% DNase); after enzyme removal the cells were washed three times and thereafter incubated in Eagle's MEM + 20% foetal calf serum at 37 °C in the presence of 10% CO_2 for several hours. *c*, Surface immunoglobulins were evaluated by the direct immunofluorescence method. △ and ▲, IgM; ○ and ●, IgG.

Table 1 Changes in cellular composition of SJL/J thymuses with increasing age

Age	Average thymus weight (mg)	% θ-Bearing cells Mean	% θ-Bearing cells Range	% High H-2^s-bearing cells Mean	% High H-2^s-bearing cells Range	% IgG-bearing cells Mean	% IgG-bearing cells Range	% IgM-bearing cells Mean	% IgM-bearing cells Range
1 d	—	93	89–95	10	6–13	—	—	—	—
5 d	—	87	85–91	10	4–12	—	—	—	—
20 d	78	84	81–86	10	7–13	—	—	—	—
1 month	84	85	84–87	12	10–12	—	—	—	—
2 months	62	85	78–95	12	10–12	1	0–2	0.5	0–1
4 months	52	76	61–93	27	19–38	3.5	0–7	0.6	0–2
5 months	46	64	51–86	34	13–63	4.4	2–7	0.7	0–1
8–9 months	58	51	19–64	39	20–60	10.3	3–40	1.4	0.5–3
10 months	62	36	16–72	—	—	21.4	5–61	6.0	0–18
11 months	68	31	11–89	58	37–79	21.3	0.5–50	4.1	0–10
15 months	140	23	8–40	—	—	50.2	30–63	21.8	10–35

Percentages of θ-bearing cells and high H-2^s-bearing cells were determined by cytotoxic testing (trypan-blue dye-exclusion). Anti-θ C3H serum (prepared by six weekly injections of 10^7 C3H thymus cells into AKR/J mice) or H-2 alloantiserum, shown to be cytotoxic only to thymocytes having high levels of H-2 antigen (obtained following six weekly injections of 10^8 SJL/J spleen cells into C3H mice), were used. Diluted antiserum (1:2–1:8) was added to 10^6 thymus cells; the mixtures were incubated for 20 min at 37 °C and after several washings with tyrode-BSA (0.1%) complement was added and the cells were further incubated for 30 min at 37 °C. The percentage of IgG and IgM-bearing cells was determined by direct immunofluorescence[7] with fluorescein-conjugated goat anti-mouse IgG or IgM sera (Meloy). Double labelling evaluation was carried out as follows. Thymus cells from 12-month-old SJL/J mice were incubated with fluorescein labelled anti-mouse Ig serum, the excess of serum removed from the cells by repeated washings and the cells treated with AKR anti-θ C3H serum followed by incubation with rhodamin-labelled anti-mouse IgG (Miles-Yeda, Israel). The cells were then tested under the fluorescence microscope. The immunoglobulin-bearing cells had a green yellow fluorescence (mostly caps), whereas the θ-carrying cells had a red fluorescence (mostly rings). No double-labelled cells were observed and the percentage of immunoglobulin and θ-bearing cells corresponded with the values obtained when each marker was tested separately.

received a single intraperitoneal injection of SRBC (0.5 ml 10% suspension) and 5 d later the plaque-forming capacity of thymus cells estimated[8] (mean values 1,415 ± 289 plaques per thymus). Antibody production by thymus cells was reported to occur only following thymus injury caused by antigen injection into the thymus[9].

Thymocytes bearing low levels of θ antigen and high levels of H-2 are considered to be the immunocompetent cells able to cause a GVH response. We therefore tested whether the age-related increase within the thymus of the H-2 population could be correlated with an increased capacity of T cells derived from older mice to induce a GVH response. Thymus cells(10^7) from 2,9 and 15-month-old SJL/J mice were injected into 10-d-old (SJL/J × C57BL/6) F_1 hybrid mice (8–10 mice per group) and estimating their spleen index 10 d later. A statistically significant increase in the ability of thymocytes from 9-month-old compared with 2-month-old mice to evoke a GVH response was indicated (spleen indices being 2.76 ± 0.10 compared with 1.71 ± 0.14, $P \ll 0.01$; thymus cells from 15-month-old mice yielded spleen indices of 2.35 ± 0.28; $P < 0.1$ for thymus cells from 2-month-old compared with 15-month-old mice). The results suggest that the θ-positive, high H-2 immunocompetent population is relatively and transitionally enriched in the thymus of 9-month-old animals, whereas in the 15-month-old thymus this population is reduced.

A certain thymus subpopulation within the 5–12 month age range could not be classified as T or B cells. The possibility that these cells may still be in an undifferentiated state and could thus differentiate into haematopoietic colony-formers when inoculated into lethally irradiated syngeneic mice was tested. Increasing numbers of thymocytes, taken from 2, 9 and 12-month-old SJL/J mice, were injected intravenously into irradiated mice and the occurrence of spleen colonies evaluated. An age-related increased ability of thymus cells to form colonies was noted (Fig. 2). Thymocytes from other strains of mice when injected in similar conditions cause the development of very few colonies[10].

Following the normal pattern of thymus growth and age-dependent atrophy[11] a secondary increase in thymus weight was observed in SJL/J mice from 7–9 months onwards (Table 1). This weight increase seemed to coincide with thymus repopulation by B cells. The thymus cells at this stage were hydrocortisone-resistant. The cellular composition and weight of 12-month-old thymuses were not altered following hydrocortisone treatment, whereas in the 2-month-old mice the hydrocortisone caused thymocyte destruction expressed in the marked drop of thymus weight and decreased incidence θ-bearing cells (Table 2).

Thus, with age increase the thymus of SJL/J mice includes

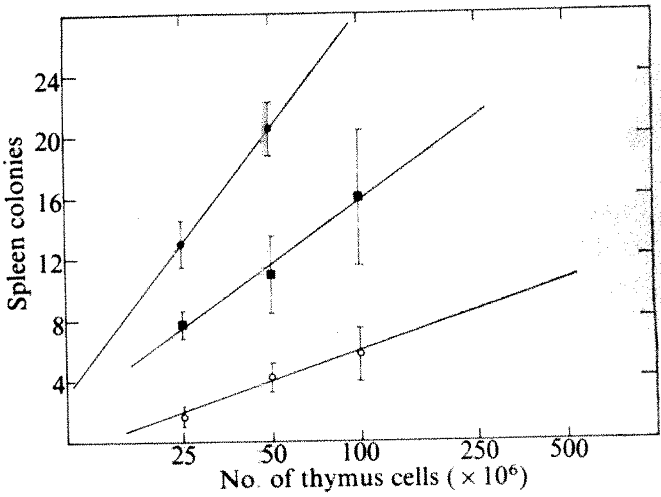

Fig. 2 Spleen colony-forming capacities of thymus cells from SJL/J mice of different ages. Increasing numbers of thymocytes (25 × 10^6–100 × 10^6) from 2(○), 9(■) and 12-month-old SJL/J mice (●) were injected intravenously into irradiated (750 rad whole-body exposure) SJL/J mice. Spleen colonies were counted 8 d later, after fixing the spleens in Bouin's solution (mean ± s.e.).

Table 2 Hydrocortisone effect on thymus weight and cellular composition of young and old SJL/J mice

Age (months) and treatment		Thymus weight (mg)	% θ-bearing cells	% high H-2-bearing cells	% Ig-bearing cells
2	Normal	57.3 ± 2.25	90.5 ± 0.37	16.5 ± 3.28	NT
	Hydrocortisone	11.7 ± 0.82	23.8 ± 4.07	91.8 ± 1.55	NT
12	Normal	59.5 ± 17.55	32.0 ± 6.58	62.0 ± 7.8	25.8 ± 7.49
	Hydrocortisone	49.3 ± 5.00	26.5 ± 3.49	89.3 ± 3.14	35.8 ± 4.02

SJL/J mice, 2 months old and 12 months old, were treated intraperitoneally with 2.5 mg hydrocortisone 48 h before they were killed, and their thymuses evaluated. Percentage of θ-bearing cells and high H-2-bearing cells was determined by cytotoxic testing, and the Ig-bearing cells were determined by direct immunofluorescence[7].

NT, not tested.

immunocompetent T and B cells (the influx of B cells being responsible for the secondary increase in thymus weight), and therefore possesses the cellular constitution and functional abilities of a peripheral lymphoid organ. The depletion of θ-bearing cells (from the age of 8 months onwards) and the occurrence of IgG and IgM-bearing cells in the thymus may be related to unique observations specific for the SJL/J strain of mice. Since immune functions involve the participation of thymus-derived lymphocytes, the comparative early decrease in the immune reactivity of SJL/J mice may be related to a 'defect' in a thymus 'regulating factor'. The susceptibility of SJL/J mice to develop B lymphatic leukaemia, following chemical carcinogen treatment[12], could perhaps be correlated with the unique capacity of B cells to home in the thymus. Debatable information has been provided concerning the presence of some B cells in the thymus of NZB mice[6,13]. Recently[14], it was reported that in the thymus of NZB/NZW mice immunoglobulin positive cells were observed at the age of 54–65 weeks.

This work was supported by a grant from the Leukemia Research Foundation, Chicago, Illinois.

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Fusion of hen erythrocytes with yeast protoplasts induced by polyethylene glycol

POLYETHYLENE glycol (PEG) has been used to induce the fusion of protoplasts of higher plants[1,2]. Ahkong *et al.*[3] have also observed that PEG will cause hen erythrocytes to fuse to form binucleate and multinucleate cells. The availability of a common chemical agent that can be used effectively for the fusion of both animal cells and plant protoplasts has prompted us to investigate the possibility that PEG may likewise induce interspecific fusion between hen erythrocytes and yeast protoplasts.

Hen erythrocytes (Fig. 1*a*) and yeast protoplasts (Fig. 1*b*) were mixed in the cell ratios 1:250, 1:50, 1:10, 1:1 and 10:1, erythrocytes:protoplasts (approximately 6×10^8 cells in total). An appropriate volume of the protoplast preparation was centrifuged (1,000*g* for 3 min) and the supernatant removed. The appropriate volume of erythrocyte preparation was then added gently to the protoplast pellet and, after a brief centrifugation (800*g* for 5 min), the supernatant was carefully removed. The erythrocytes and protoplasts were resuspended and thoroughly mixed in 1 ml 40% w/v PEG solution (molecular weight 6,000; Koch-Light) in the HEPES-buffered Eagle's medium, *p*H 7.4, at 37° C, and then incubated at this temperature for 15 min, during which time the erythrocytes and protoplasts were observed to form nonspecific aggregates. The incubated suspension (1 ml) was diluted with 5 ml of the buffered Eagle's salt solution at 37 °C and, after centrifugation (800*g* for 5 min), the cells were resuspended in 1 ml of the same solution at 37 °C. At this stage, aggregation was less and the cells were more loosely associated. On continued incubation, the erythrocytes became swollen and cell fusion was observed by light microscopy. Samples were removed for electron microscopy at intervals of up to 1.5 h. These were fixed in 3% v/v glutaraldehyde (1 h at 22 °C) in buffered Eagle's salt solution and then treated as described previously[7].

Small variations from the above procedure, such as the use of yeast cells in stationary phase, markedly diminished the yield of fused cells. With the method described, approximately 0.1% of the erythrocytes were involved in interspecific fusion when the erythrocytes and yeast protoplasts were mixed in the proportions of 1:1 or 1:10 (Fig. 2*a*). With a cell ratio of 10:1 (erythrocytes:protoplasts), interspecific fusion was reduced and multinucleated erythrocytes were formed in relatively high numbers (Fig. 1*c*). With ratios of 1:50 or 1:250, very large protoplasts were seen, presumably the product of PEG-induced fusion of yeast protoplasts (Fig. 1*d*). Some of these giant protoplasts contained a few large, intracellular vacuoles, indicating that fusion of vacuoles had also occurred.

Further evidence for the occurrence of interspecific fusion was provided by electron microscopy (Fig. 2*b*). The erythrocyte plasma membrane seems to fuse with the yeast protoplast

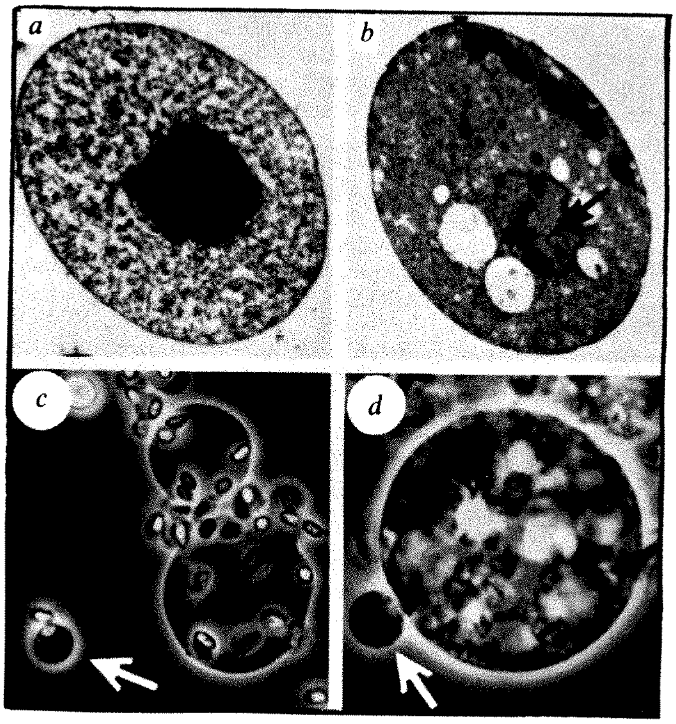

Fig. 1 Electron micrographs of (*a*) nucleated hen erythrocyte and (*b*) freshly isolated yeast protoplast showing nucleus (arrow), (×7,980). Hen erythrocytes were prepared as described previously, and suspended in a modified Eagle's basal salt solution[4], buffered at *p*H 7.4 with 20 mM HEPES buffer (approximately 6×10^8 cells ml^{-1}). Yeast cells, *Saccharomyces cerevisiae* strain NCYC 0263B, were cultured[5], and yeast protoplasts were obtained from cells in exponential growth using helicase[6]. The protoplasts were centrifuged (1,000*g* for 15 min) in a discontinuous density gradient of Ficoll 400 (Pharmacia, Uppsala, Sweden) dissolved in an osmotic stabiliser (0.7 M sorbitol, 0.01 M citric acid, *p*H 6.5); the osmotic stabiliser (5 volumes) was layered on 5% w/v Ficoll (8 volumes), which in turn was above a layer of 8% w/v Ficoll (8 volumes). The sedimented protoplasts were resuspended in the osmotic stabiliser (approximately 6×10^8 protoplasts ml^{-1}) and used immediately. *c*, Multinucleate erythrocytes formed by PEG treatment of erythrocytes and yeast protoplasts mixed in a cell ratio of 10:1. A single unfused erythrocyte (arrow) is shown for comparison (light micrograph, ×475). *d*, Giant yeast protoplast formed by PEG treatment of a mixture of erythrocytes and yeast protoplasts containing a large excess of protoplasts (cell ratio of 1:250) observed after incubation for 1 h in Eagle's medium at 37 °C. A single unfused yeast protoplast (arrow) is shown for comparison (light micrograph, ×950).

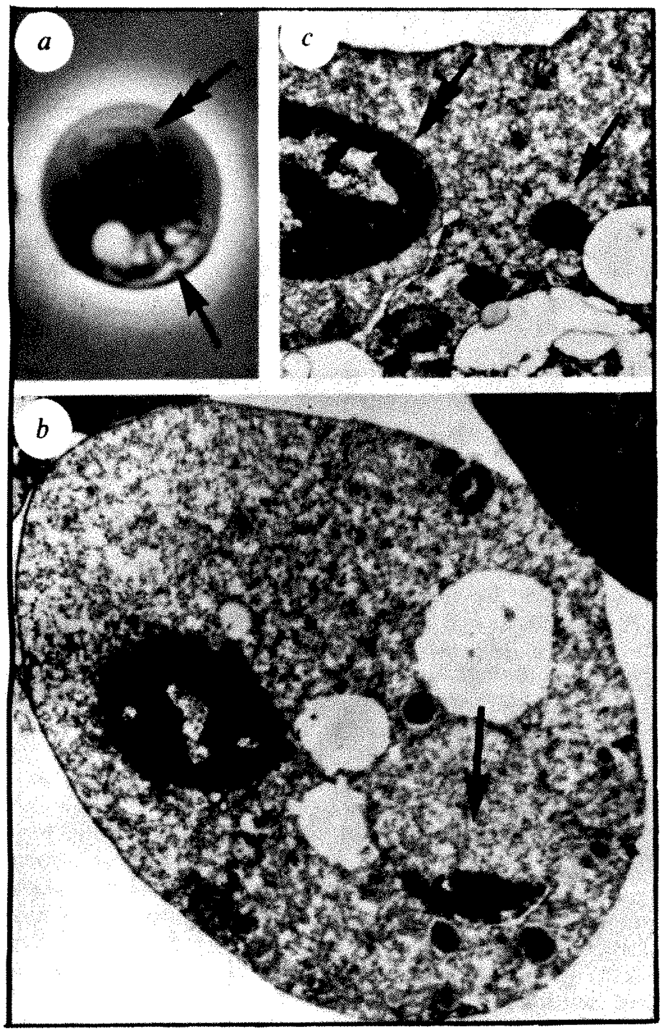

Fig. 2 *a*, Erythrocyte–yeast protoplast heterokaryon (yeast protoplast component—single arrow; erythrocyte component—double arrow) formed after PEG treatment of erythrocytes and yeast protoplasts mixed in the proportions of 1:10 observed after incubation for 40 min in Eagle's medium at 37 °C (light micrograph, ×618). Electron micrographs of (*b*) erythrocyte–yeast protoplast heterokaryon showing both the erthrocyte nucleus and the yeast nucleus (arrow), (×12,540) and (*c*) formation of a broad cytoplasmic bridge between an erythrocyte (double arrow) fusing with a yeast protoplast (single arrow), (×14,060).

plasma membrane, and as a result, broad cytoplasmic bridges are formed between adjacent cells (Fig. 2*c*).

The fact that the plasma membranes of cells from genera as widely separated as hen and yeast can fuse in appropriate experimental conditions is of particular interest, and studies are in progress to see if it will be possible to culture the heterokaryons obtained. These investigations may well further our knowledge of membrane structure, and of nuclear and cytoplasmic interactions in such animal–fungal heterokaryons. In addition there are many fundamental morphological and biochemical problems that could be investigated with the aid of interspecific fusion of this kind.

This work was supported by a grant (to J.A.L.) from the MRC.

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Visual control of head movements during avian locomotion

THE impression that pigeons, chickens, and many other common birds bob their heads backward and forward as they walk is compelling but illusory. In reality, the walking bird thrusts its head forward, then holds it nearly stationary while the neck retracts and the body catches up. The head moves backward and forward with respect to the body, but moves only forward in the environment. Although this was demonstrated on film more than 40 years ago[1], theorists concerned with the evolution of visual systems[2–4] continue to suggest that a back and forth oscillation of the head is important in avian vision.

The rhythm of head movement may, by a reflex action, aid balance or it may become entrained by other endogenous rhythms during walking[5]. Since vestibular stimulation can induce rhythmical head rotations[6] and since doves' linear head movements have the same rhythm as head rotations[7], stimulation of the labyrinths during locomotion may elicit the periodic head movements. Finally, since rotations of the visual field induce periodic optokinesis in many species[8] visual displacement may also be responsible for rhythmical linear head movements. A series of experiments were carried out on the Ring Dove (*Streptopelia risoria*) to dissociate the three factors most likely to control head movements during locomotion: movement in the visual environment; acceleration in the inertial (labyrinthian) environment; and the mechanics of stepping.

A dove was filmed from above as it walked about and fed in a cylindrical cage (Fig. 1*a*) and successive single frames of the recording were analysed. The normal pattern of horizontal head movement was determined relative to the cage, to the camera support, and to the bird's body. As a control for the effects of the apparatus used in later experiments, a small plastic platform was temporarily glued to the feathers on the bird's back and a rod (held vertical by a counterbalanced thread) was loosely attached to a pivot on the platform (Fig. 1*b*). When the dove was recorded in the cage, the effects of the platform and vertical rod on feeding behaviour were negligible and the patterns of head movement were normal.

A duplicate cage was constructed from extremely lightweight material. The entire cage weighed 180 g, approximately the same as the weight of a dove. The cage was supported over a level plate by hundreds of miniature ball bearings. The platform on the bird's back was attached by a counterbalanced joint to a rigid column fixed below the camera (Fig. 1*c*). Without supporting the bird, the joint permitted the vertical displacements and pitch and roll movements normal in walking, but restrained the bird's body from any horizontal linear displacements or

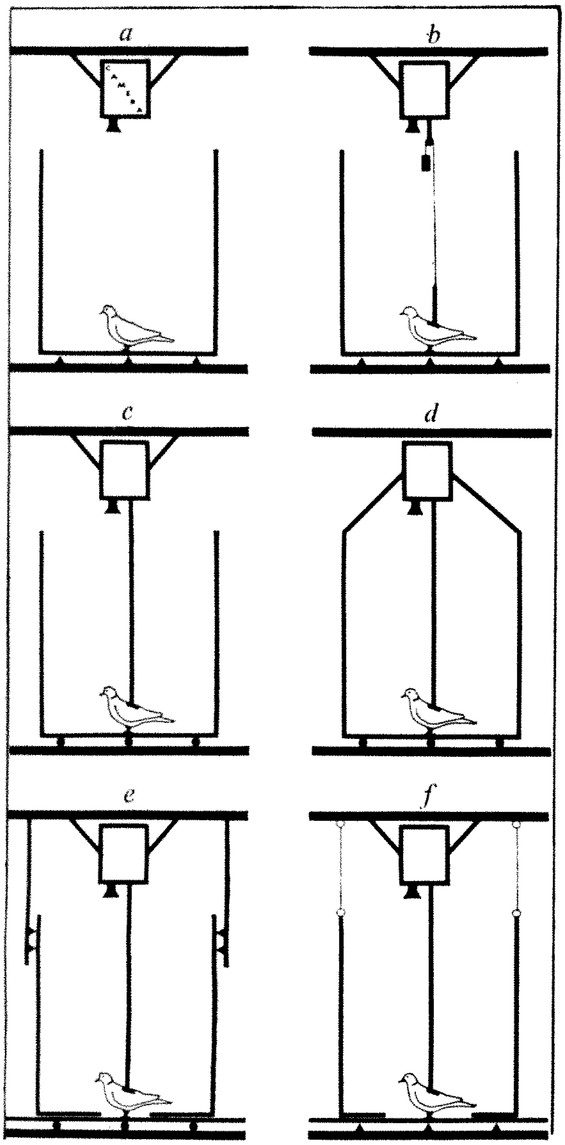

Fig. 1 *a–f* The six experimental conditions discussed in the text. A television or cine camera was mounted on a frame vertically above a cylindrical cage 50 cm in diameter with a 60 cm high wall. ▲, Fixed cage support; ●, ball bearing cage support. The sliding cage was constructed from expanded polystyrene (2 mm thick) with the underside of the floor and lower wall reinforced by 0.5 mm cellulose acetate. The ball bearings were 300–400 µm polystyrene beads. Three birds participated in all six phases of the experiment and three others participated in only the first three phases. Each day each bird was tested in only one cage configuration. The floor and walls of the cage were distinctly patterned and a variety of seeds were scattered on the cage floor. In the first four phases, the bird was allowed 1 h to adapt to the cage before fresh seeds were added and the recording started. In the last two situations recording was started soon after the bird was placed in the cage.

rotary movements. Thus, when the bird took a step, rather than moving across the floor, the floor moved under the bird. Similarly, rather than turning in the cage, the cage rotated around the bird. The movements of the floor and walls relative to the bird were the same as would have occurred in free movement.

When recordings of the bird walking and feeding in this cage were analysed, head movements were normal with respect to the visual world (the cage) and the bird's body, but abnormal with respect to the inertial world (the camera supports). Whereas the head continued to be alternately thrust forward and held still in visual space, in inertial space it moved backward after each forward thrust. This backward movement in the inertial frame of reference would not normally take place during locomotion.

The camera was then detached from its fixed supports and attached to the moveable cage (Fig. 1*d*). The cage was pushed forward or rotated by hand at speeds approximating the bird's normal rate of travel. This would show if progression through inertial space could be sufficient to sustain periodic head movements. The dove's head stayed locked to its visual world and did not oscillate with respect to the body or surge in inertial space.

To determine the role of stepping movements, the camera was remounted on its fixed support and the cage rigidly attached to the same framework. A large false floor, adjusted to equal the dove's own weight, was mounted on bearings immediately below the real cage floor which had a hole cut in it. The bird was placed in the cage on the false floor and attached to the joint assembly (Fig. 1*e*). Recording was started and continued until spontaneous walking began. If this did not occur within 15 min, walking was induced by gently pulling on the false floor. The experiment was repeated three times with each dove and in each case the bird either extended its neck gradually forward and started running until it thrust the false floor out from underneath it or squatted and started peering rapidly around. There was no evidence of the 'bobbing' of the head observed during normal locomotion, although the doves were obviously reacting to the absence of visual feedback from their stepping.

In a complementary test, the hole in the cage floor was enlarged and the cage was suspended by thread from the ceiling. The false floor was fixed (Fig. 1*f*). After the bird was attached to the camera column, the cage was moved by hand at approximately the same speed used in condition (*d*). When the cage was rotated, the bird showed the classical optomotor response[6,8] of the head: alternately stabilising in visual space and saccadically moving to a new position. When the cage was moved smoothly in a rostral to caudal direction (up to 20 cm displacement), the bird's head was alternately stabilised and thrust forward in its visual space while it oscillated back and forth with reference to the body and inertial space.

Conditions *a* and *b* established the range of normal patterns of head movement shown by doves in the cylindrical cage and showed that having a vertical rod attached to the bird's back did not alter the normal pattern of head movement. Condition *c*, in which the cage moved about the bird rather than the bird about the cage, demonstrated that progression through inertial space is not necessary for the normal pattern of head movement. When the whole cage was moved with the bird fixed in the cage (condition *d*), it showed that progression through inertial space is not sufficient, in itself, to induce a normal periodic pattern of head movement. Condition *e* demonstrated that the mechanics of stepping are not sufficient to generate the periodic head movements normally seen. Condition *f* showed that the mechanics of stepping are not necessary for the normal pattern of head movement but that displacement through visual space is a sufficient condition. These results hold both for linear and angular movements.

These experiments demonstrate the primacy of vision in regulating periodic eye and head movements in the dove. The rhythmical alternation of visual stabilisation and rapid reorientation described here is not limited to walking birds with dove-like head movements[9]. Many long-necked birds rhythmically dart their heads about while inspecting possible food sources. Some of those with shorter necks and large beaks show saccadic exploratory eye movements. Hopping birds with short necks have, by necessity, a saltatory mode of head and eye movement. That birds of diverse shape and mode of locomotion show similar patterns of head and eye movement should be important to theories of the evolution of visual perception[10] and suggests that the neural substrate of this mode of visual exploration is very conservative during avian evolution.

This work was carried out while the author was a postdoctoral fellow in the Department of Psychology of the University of Edinburgh working with Dr C. Trevarthen and Professor D. M. Vowles, and was supported by a US NIMH fellowship and by an SRC research grant to Professor Vowles.

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Visual detection analysed in terms of luminance and chromatic signals

THE ability to discriminate surfaces of different colour and luminance is a basic property of human vision. This has been studied by determining visual thresholds for test stimuli of one colour superimposed on a background of another colour. Stiles has suggested that the observer samples the responses to the test flash from a number of 'mechanisms' each of which adapts independently to the background[1,2]. In the simplest form of the model, the responses of the different mechanisms to the test flash are also treated independently, that is, a flash is seen if it exceeds the threshold criterion of any of the mechanisms. The spectral sensitivities of Stiles' mechanisms (to both test and background stimuli) are similar to the absorption and action spectra of the three normal types of human cone[3-5]. Thus, as a simple approximation, visual mechanisms correspond to individual classes of cone and they adapt and are sampled independently; this scheme would be consistent with the demonstration of adaptation occurring within monkey cones[6].

This simple model has proved to be of great value under a wide range of adaptation conditions[1,2], but certain objections have been raised. Electrophysiological studies have shown that the output from the cones is recoded in terms of luminance and colour information at an early stage[7-9]; it is therefore not easy to see how the outputs from different cone types can be sampled independently. Psychophysically, Stiles has shown that up to seven mechanisms must be proposed to explain his data[2], although there are thought to be only three types of cone. The spectral sensitivity of at least one of Stiles' mechanisms (π_5) cannot correspond to a cone action spectrum, as can be shown from colour matching data[2]. The model cannot explain the inhibitory interactions which may be demonstrated when different colours of test flash are superimposed[10,11]. It is also inconsistent with the 'supersummation' effects observed when different colours of background light are added together[12]. Finally, the model predicts that the spectral sensitivity for a test flash on a white background should correspond to an upper envelope of the sensitivities of the three cone types and so should have a rather broad maximum in the region 540–570 nm; however, the observed sensitivity curve (Fig. 1, ○) shows relatively sharp peaks at about 610 and 530 nm which cannot be fitted by Stiles' mechanisms or by the cone action spectra[13].

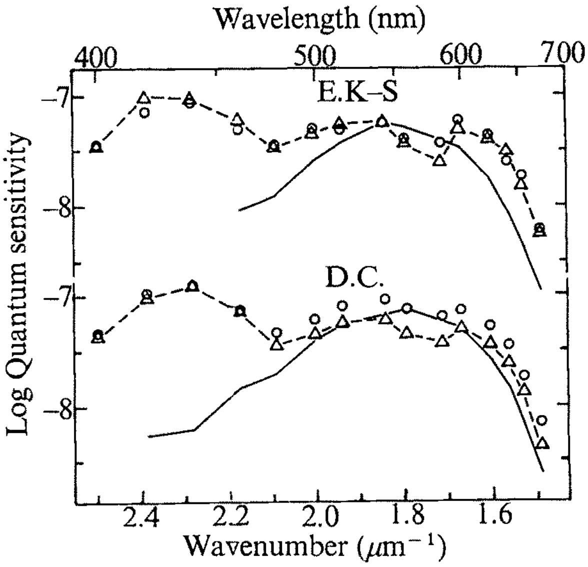
To overcome some of these problems, Sperling and Harwerth[13] have proposed that there is a general linear subtractive interaction between the outputs of the 'red' and 'green' sensitive cones, that is, the red cone response to a test flash is reduced in proportion to the green cone response and vice versa; in this way, they could fit the spectral sensitivity data for a white background. This subtractive process could presumably form the basis for the inhibitory interactions between test stimuli mentioned above[10,11]. It may also correspond to the red–green opponent colour responses proposed by Hering (1875) and by Jameson and Hurvich[14] and which have been demonstrated in single units of the monkey visual system[7-9].

According to Hering's classical theory, the opponent colour channel is concerned with the signalling of chromatic as opposed to luminance information. Thus, if the opponent colour system is indeed responsible for visual detection of spectral flashes on a white background, the colour of the test light may be expected to be observable at the detection threshold. This prediction has been tested with a two-channel Maxwellian view apparatus[15] using a 1° 200 ms test flash on a white background (Fig. 1).

Although detection sensitivity tends to be slightly higher than colour sensitivity throughout the red–green range of the spectrum, there is reasonable agreement between the two sensitivities, supporting the idea that opponent colour mechanisms are mainly responsible for detection in this situation. This conclusion is further supported by the observation that red and white test stimuli at their respective detection thresholds are as easily discriminated from each other as each is discriminated from a blank stimulus (P. E. K-S., unpublished); the colour of the red stimulus is thus detectable at threshold. This observation is consistent with other psychophysical studies[16,17].

A surprising aspect of this interpretation is that no contribution from the luminance signalling system[7-9,14] has been assumed; one may expect this to have contributed a relatively broad peak near 550 nm. I believe that the lack of evidence of

Fig. 1 Spectral sensitivity curves determined from thresholds for a circular 1° 200 ms foveal test flash presented on a 4° white background of 1,000 trolands (luminance in candelas m^{-2} × area of pupil in mm^{-2}) and about 3200 K colour temperature. For the two subjects, E. K-S. (age 34) and D.C. (age 31): ○, detection thresholds; △ and dashed lines, thresholds for determining the colour of the test stimulus; continuous curves, sensitivity of the luminance channel to the test stimulus (the shapes of these curves were derived from the thresholds for detecting 25 Hz flicker on the same white background, whereas the vertical positions were derived from the corresponding curves in Fig. 2). Test wavelengths were presented in random order; thresholds were determined by subject setting, and the plotted points correspond to means of three or more determinations (s. e. typically 0.04 log units). Sensitivity is expressed as reciprocal quanta s^{-1} degree^{-2}.

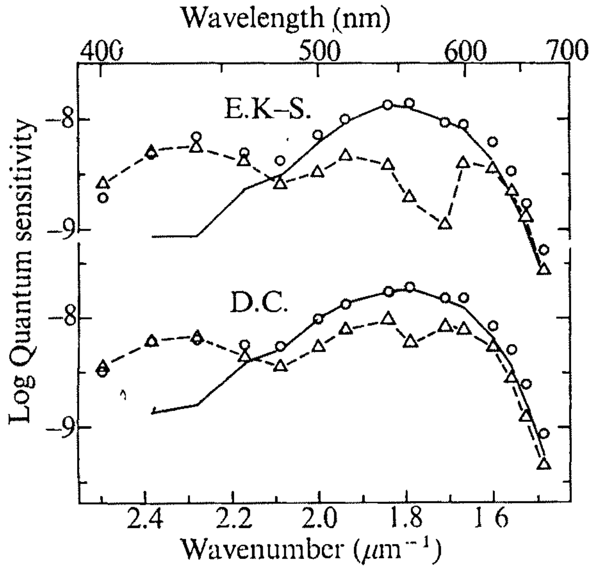


contribution from the luminance system is caused by the low sensitivity of this system in the conditions of Fig. 1, perhaps for three reasons. First, the relatively long duration (200 ms) of the test flash in Fig. 1 favours the opponent colour system relative to the luminance system because of the greater temporal integration in opponent colour channels[18,19]. Second, it can similarly be argued that the relatively large (1°) test flash may again favour the opponent colour system which has a greater spatial integration than the luminance system[20,21]. Third, it is possible that the white background, which is a strong stimulus for the luminance but not for the opponent colour system, may cause a corresponding reduction in sensitivity, specifically within the luminance channel.

It is possible to test these ideas by altering the experimental conditions in the ways expected to favour the luminance channel. For example, the luminance of the white background may be reduced in steps and finally to zero (absolute foveal threshold); when this is done, there is a tendency for the two peaks in the red–green range to become less prominent and, as predicted, a broad peak near 550 nm becomes apparent[13,22]. Use of a very small test spot (0.05°) on the white background of Fig. 1 also yields a smoother sensitivity curve with a broad peak near 560 nm (P. E. K-S., unpublished).

The third alternative procedure used for favouring the luminance channel, that is by reducing the test exposure to 10 ms, is illustrated in Fig. 2. A relatively broad maximum of detection sensitivity at 550–560 nm is found for both subjects. Similar measurements have been reported by Ikeda and Boynton[23]. The interpretation of this maximum as the contribution of the luminance channel is supported by two observations. First, the colour sensitivity (Fig. 2) is considerably lower than the detection sensitivity in this region of the spectrum (particularly for subject E. K-S.). Secondly, the sensitivity of the luminance system may be estimated by determining the subject's thresholds for detecting high frequency (25 Hz) flicker on the same white background; the opponent colour channels should contribute little to these data since they are known to have a poor response to high frequency flicker[18]. The continuous curves in Fig. 2 are derived in this way and fit well the central region of the detection sensitivity curves (about 500–600 nm for E. K-S. and about 480–580 nm for D.C.).

Fig. 2 Spectral sensitivity curves for detection (○) and for colour (△ and dashed line) as in Fig. 1 but for a 10 ms instead of a 200 ms test flash; continuous curves, sensitivity of the luminance channel determined from 25 Hz flicker thresholds (these curves have been shifted vertically to match the detection sensitivity curve near 550 nm). To a good approximation, detection sensitivity corresponds to colour sensitivity or luminance sensitivity, whichever is the greater.



It is now possible to understand why there is no evident contribution from the luminance channel for the 200-ms test flash (Fig. 1). For the white background (Figs. 1 and 2), the subjects were about 0.6 log units more sensitive to a 200-ms than to a 10-ms white test flash. Thus, we may expect the luminance channel to be 0.6 log units more sensitive for the 200-ms test flash of Fig. 1 than for the 10-ms test flash of Fig. 2, and the continuous curves of Fig. 1 are the corresponding sensitivities of the luminance channels derived in this way. The luminance sensitivity (Fig. 1) is less than the colour sensitivity except for a restricted region around 570 nm and this explains why the effect of the luminance system is not evident for the detection of a 200-ms test flash.

In summary, it is proposed that a test flash is detected either by its luminance or by its colour; this conclusion is consistent with recent psychophysical experiments[11,24,25]. Thus, the spectral sensitivity for the detection of a test flash (Figs. 1 and 2) corresponds approximately with the upper envelope of the colour sensitivity and the luminance sensitivity. The results indicate that there may be some probability summation between the two systems when they have nearly equal sensitivity (for example, for 10-ms red test flashes, Fig. 2). It is probable that the major part of light adaptation occurs within the cones[6], but it may also be necessary to consider adaptation within the luminance and opponent colour channels. Thus some adaptation in the photopic system may take place at a point where the signals from receptors have been 'pooled'; Rushton[26] has demonstrated the importance of an adaptation pool in the scotopic system. Finally, we do not have direct access to the outputs from different classes of cones; we can only sample this information indirectly after it has been reprocessed in terms of luminance and colour information.

A final comment is needed about the relation between the opponent colour and luminance systems. It is quite possible that, in some situations, opponent colour cells may be involved in the transmission of luminance information. For example, the outputs from red-on-centre and from green-on-centre geniculate cells[8] could be combined in the cortex to form a unit with luminance-type properties; the present experiments do not rule out this possibility.

This work was supported by a grant from the Royal Society and by a research grant from the SRC. I thank Mr D. Carden for assistance and for acting as subject, and W. S. Stiles, R. M. Boynton, P. Gouras, D. Regan, C. F. Stromeyer, J. R. Cronly-Dillon, J. J. Kulikowski and D. Carden for their comments on the manuscript.

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Received February 19; accepted March 9, 1975.

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Sensitivity of self-potentiating effect of luteinising hormone-releasing hormone to cycloheximide

THE hypophysiotropic luteinising hormone–releasing hormone (LHRH) has a number of distinct actions on the anterior pituitary gland. In addition to stimulating the release and increased synthesis of gonadotrophins[1], this peptide also has a priming or self-potentiating effect so that intermittent stimulation of the pituitary with identical doses of LHRH causes a progressively increasing series of responses of luteinising-hormone (LH) secretion. Self-potentiation by LHRH has been observed *in vivo* in rats[2] and man[3] and may play an important role in the generation of the LH surge which precedes ovulation. The effect can be obtained[4] following repeated, brief exposures of the isolated, perifused rat pituitary gland to LHRH and the time course of development and decay of the potentiated state in these conditions is similar to that observed in the intact rat[2]. We have investigated the effects of an inhibitor of protein synthesis on this phenomenon and report here that cycloheximide suppresses the priming effect of LHRH without impairing the acute response of LH release.

Pituitary glands from four dioestrous female rats were halved and divided into two groups of paired halves. Each group was placed in an identical Millipore Swinnex filter chamber and perifused simultaneously at 37 °C with Krebs–Ringer bicarbonate medium (*p*H 7.4) containing bovine serum albumin (2.5 g l^{-1}), glucose (2 g l^{-1}) and gassed with 95% O_2–5% CO_2 (v/v). The flow rate was maintained at 0.2 ml min^{-1} by means of a peristaltic pump and the effluent medium was collected every 10 min (2 ml fractions) and assayed for LH using an NIH radioimmunoassay kit.

Cycloheximide (5 μM) was added to the medium used to perifuse one group of halved pituitaries whereas the other group received only the control medium. After an initial period of 100 min in which the secretion of LH was allowed to decline towards basal levels, both groups of halved glands were subjected to 5 mins perifusion with 10 ng of synthetic LHRH dissolved in 1 ml of perifusion medium, introduced by transferring the inlet tube of the system to the test sample. Identical challenges with LHRH were administered at intervals of 1 h for a further period of 4 h and the effects on LH secretion in both groups are shown in Fig. 1.

Control and cycloheximide treated groups both showed similar patterns of LH secretion during the preliminary period of perifusion and also in response to the first stimulation with LHRH, which was given before LH secretion had reached basal levels. With subsequent exposures to LHRH, however, only the hemipituitaries perifused with control medium showed the typical pattern of increasing responsiveness to LHRH and after five such challenges the rate of secretion of LH had risen to about five times that of the glands treated with cycloheximide. The latter did not exhibit a potentiated response and a series of equal peaks of LH secretion were obtained in the presence of 5 μM cycloheximide.

Inhibition of the self-potentiating effect of LHRH has been consistently obtained in similar experiments in which concentrations of cycloheximide ranging from 5 μM to 250 μM were used. In no case was the acute release of LH in response to LHRH abolished. Dissociation between the acute release of LH and the development of enhanced responsiveness to a series of LH-releasing stimuli has also been obtained in preliminary experiments in which isolated perifused pituitary glands were exposed for 5 min to medium containing 50 mM K^+. Osmolarity was maintained by adjusting the Na^+ concentration and the stimulus was administered at 1-h intervals. The high potassium medium caused an acute release of LH similar to that observed with LHRH but, as in the presence of cycloheximide, the series of LH peaks were of equal magnitude and did not exhibit the increased responsiveness shown by glands primed with LHRH.

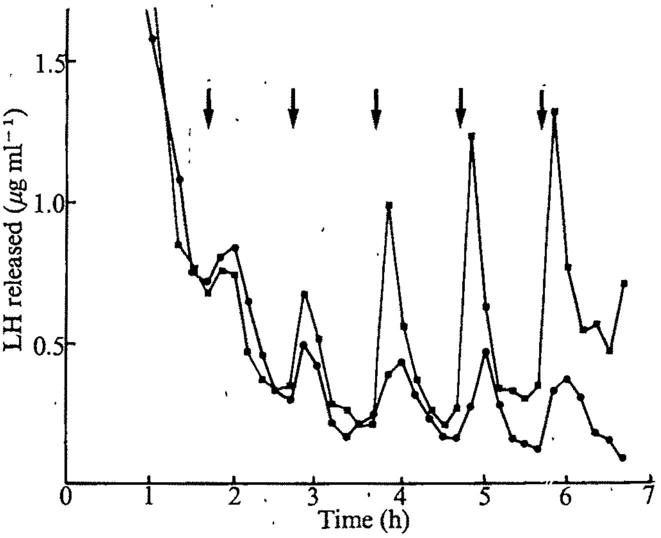

Fig. 1 Effect of cycloheximide on stimulation of LH release by LHRH. Two groups of paired, halved rat adenohypophyses were simultaneously perfused with Krebs–Ringer bicarbonate medium alone (■) and containing 5 μM cycloheximide (●). Glands were exposed to synthetic LHRH (10 ng ml^{-1}) for 5 min at the times indicated by arrows. LH released to the perifusion medium in 10 min intervals was measured by radioimmunoassay and results are expressed in terms of the NIAMD-rat LH-RP-1 reference preparation. Cycloheximide abolished the self-potentiation produced by LHRH without reducing the acute release of LH.

These results show clearly that the augmented release of LH which occurs from the primed pituitary gland represents a specific response to the hypophysiotropic hormone rather than a nonspecific sequel to the discharge of LH. Release of LH evoked by LHRH in the presence of cycloheximide or induced by depolarising levels of K^+ does not cause a potentiated response to further stimulation. The priming effect would seem to involve the synthesis of new proteins and it has been suggested that increased synthesis of LH may be important[3]. Only a small percentage of the LH content of the gland is, however, released in response to acute stimulation with a supramaximal dose of LHRH. An intermittent exposure to LHRH in the presence of cycloheximide does not cause a diminution of the acute secretory response. It is likely that the availability of LH is not a limiting factor and that the synthesis of some other component of the release mechanism may be essential for self-potentiation to occur. The effects of LHRH and cycloheximide on the incorporation of labelled amino acids into LH and various subcellular components are being studied and should resolve this issue.

This work was partly supported by an MRC programme grant. We thank C. O'Neil and Mrs J. Pennington for technical assistance. Rat LH immunoassay materials were provided by the NIAMD–NIH rat hormone distribution programme and the LHRH was donated by Roche Products Ltd.

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Inhibition of airlicking in thirsty rats by cooling the preoptic area

THIRSTY rats lick avidly and persistently at a stream of air flowing through a drinking tube[1], behaviour which is not dependent on the previous experience of drinking water through a drinking tube[2] or on olfactory cues[3]. The most plausible explanation for the rewarding property of airlicking is that it is a result of evaporative cooling of the orolingual region. Heating and humidifying the air so as to decrease its cooling power reduces airlicking[4]. In addition, tongue cooling alone is rewarding for thirsty rodents[5], and cold water has a greater satiating effect on water intake than warm water[6]. These observations seem to indicate that because airlicking leads to tongue cooling, it thereby reduces thirst and the reduction of thirst reinforces airlicking. Airlicking cannot be mediated entirely by tongue temperature receptors, however, because lingual denervation by bilateral section of the lingual, chorda tympani and one branch of the glossopharyngeal nerves has no influence on airlicking[7]. It is not clear whether central stimuli that affect water drinking and satiation will similarly influence airlicking. Temperature-sensitive neurones are located in the preoptic area (POA) and hypothalamus[8], and since cooling of the POA reduces water intake in thirsty animals[9], we tried to determine if cooling of the POA reduced airlicking. We found that acquisition of airlicking behaviour was completely blocked by POA cooling in naive animals and reduced about 50% in experienced animals.

Unilateral water-perfused thermodes[10] with thermistors fixed to the tips were placed stereotaxically into the POA of six naive and two experienced airlickers and into the posterior hypothalamus (PH) as a control procedure in one naive and one experienced airlicker. The animals were adult, female Sprague–Dawley rats. Of seven control animals, four were unoperated and three received either a clogged thermode, a thermistor alone, or a cannula, all in the POA.

Histology showed the POA thermodes were located close to the midline between A7470 and A8620 according to the atlas of König and Klippel[11], while those in the PH were centred at A3180.

Water deprivation for 48 h preceded the first airlicking test, and thereafter a single water ration sufficient to maintain body weight at 85% of *ad libitum* weight was given in a Richter tube immediately after each test. Food was always available except during testing. Daily, 30-min tests were conducted in a model 1316 Lehigh–Valley test enclosure modified so that a metal drinking spout could be mounted with the aperture 5 mm outside a 1.4×3.2 cm slot on the right-hand side of the front panel. An airflow of 6 l min^{-1} through the 3 mm aperture of the tube was measured continuously with a Gilmont flowmeter. Relative humidity of the air was 27–30%. The number of licks and time in contact with the tube were measured by an electronic drinkometer circuit and recorded on electromechanical counters and a cumulative recorder.

The thermodes in the POA of the naive subjects were cooled to 33 °C during the first five tests for one subject, the first seven tests for two subjects, and the first fourteen tests for three subjects and the one animal with a posterior thermode. After termination of the cooling tests, daily tests were continued for at least 14 d. The experienced airlickers were given 28 daily tests to establish stable behaviour and after 7 d of recuperation from surgery, four tests were given to assure recovery of airlicking performance. The thermodes were then cooled to 33 °C for seven tests.

The data for the control animals were pooled since there was no difference between the operated and unoperated subjects. All control animals and the subject with a PH thermode cooled to 33 °C licked persistently at the airstream during the first and all subsequent tests. The mean number of licks during the first test was 3,266 for the control animals and 3,356 for the PH subject. The animals approached an asymptotic level of licking after a few days and averaged 6,192 licks per test (range 5,090–8,024). The animal with a PH thermode averaged 7,307 licks per test.

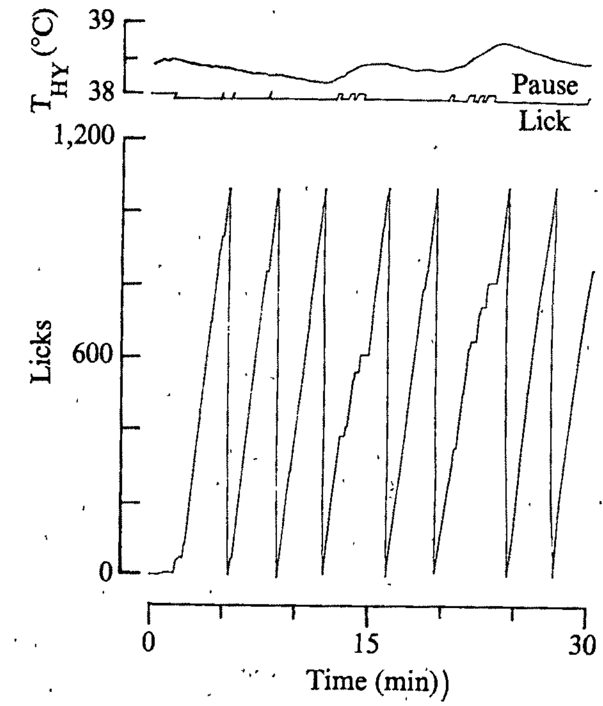

Fig. 1 Hypothalamic temperature (T_{HY}) and cumulative number of licks. The event channel (middle trace) indicates pauses in licking longer than 1.5 s.

Cooling of the POA to 33 °C precluded the acquisition of airlicking behaviour, regardless of whether cooling was applied for 5, 7 or 14 daily tests. The animals explored the cage and sampled the airstream during the first test, obtaining an average of 50 licks. During the remaining tests they rarely licked at the airstream, obtaining an average of only 11 licks per test. The airstream was not completely neutral, as the animals frequently sniffed at or to the side of the tube. This behaviour was interspersed with exploration of and sniffing at other parts of the cage as well as grooming.

The post-test rectal temperature of the control animals was 38.5 °C, whereas that of the POA subjects was consistently ($P<0.01$) elevated following tests with cooling (mean 39.6 °C, range of means 39.3–40.0 °C). Post-test rectal temperature of the subject with a posterior thermode, and of the subjects with POA thermodes when not cooled, never exceeded 39 °C. The elevation of post-test rectal temperature following cooling of the POA indicates the thermodes were in a thermally-sensitive region. It also raises the possibility that the suppression of airlicking may be a secondary effect of the elevated core temperature resulting from POA cooling. Measurement of the temperature of the PH during cooling of the POA, however, showed that a peak temperature of 40.2 °C occurred between 12 and 24 min after the start of the session. Thus, there was opportunity to airlick before the development of core hyperthermia. In addition, if it is the cooling effect of airlicking that is reinforcing[5], then the airstream should have been more reinforcing after the development of hyperthermia, which it did not seem to be. Figure 1 shows that airlicking is accompanied by a gradual fall in POA temperature. The observed rate of decrease is extremely small, of the order of 0.02–0.05 °C min^{-1} and there is an increase in temperature brought about by pauses in airlicking. During similar tests, rats airlicked for one or two hours without apparent satiation[1]. Perhaps the absence of satiation is related to the weak central cooling of airlicking in conjunction with rapid rewarming of the POA during pauses.

The animals that received cooling of the POA had difficulty acquiring airlicking behaviour after the end of the cooling tests. Two animals failed to airlick, two licked sporadically and two developed normal airlicking during the fifth test. There was no consistent relationship between the number of days of cooling and difficulty in acquiring airlicking behaviour. Perhaps the failure to develop normal airlicking immediately was a result of the subjects having learned during the cooling trials that the airstream was not rewarding.

Cooling the thermodes in the POA of two experienced airlickers for seven consecutive tests reduced airlicking an average of 40% for one subject and 53% for the second subject. Airlicking was reduced 6% for the PH subject. Thus, cooling of the POA reduced but did not eliminate airlicking once the behaviour had been acquired, whereas cooling of the PH had no substantial effect on airlicking by either the naive or the experienced subjects.

Different processes may be involved in the initiation and in the maintenance of airlicking since cooling of the POA completely blocks licking in naive but not in experienced animals. Perhaps direct conductive and convective cooling of the POA is important during acquisition of the behaviour while orolingual stimulation is sufficient to mediate airlicking once the behaviour has been acquired.

These results demonstrate that, like water drinking, airlicking by experienced rats is inhibited by cooling of the POA. Thus at least one specific central stimulus that affects water drinking affects airlicking in a similar way.

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Received January 15, accepted March 18, 1975.

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Evidence for nuclear control of amino acid transport in cultured cells

Cells incubated in an amino acid-free Krebs–Ringer buffer, or grown in medium with a reduced amino acid concentration, respond by increasing their ability to actively transport amino acids[1-3]. Studies using inhibitors of mRNA transcription and translocation suggested that this regulatory control involves mRNA transcription and the synthesis of protein intimately concerned with the transport process. We determined nuclear involvement using cells enucleated by prior treatment with the fungal metabolite cytochalasin B (ref. 4). The results presented here suggest that the nuclei of HeLa cells are important for maintaining and modifying their amino acid transport capacity in response to a reduction in the external amino acid concentration.

Growth in low amino acid BME resulted in an increase in V_{max} and a decrease in the apparent K_m value for influx. In a typical experiment, V_{max} increased from 53.51 to 70.01 pmol cm^{-2} s^{-1} and K_m decreased from 2.39 to 1.24 mM. Treatment with cytochalasin B alone did not affect glycine influx and did not prevent the transport modifications observed in normal cells. In normal cells grown in low amino acid BME (Fig. 1), glycine influx increased by 51%, whereas in enucleated cells it decreased by 48% (significant at $P<0.001$ and $P<0.02$ respectively), indicating the involvement of the nucleus in the membrane response. It seems likely that it occurs in two stages: an afferent pathway to the nucleus and an efferent pathway from the nucleus to the cell membrane. The nucleus itself, for example, could detect a lowered concentration of amino acid in its immediate environment and thus act as the initiator of the response. To test this possibility, the intracellular pool size was manipulated directly in cultured *Xenopus laevis* kidney cells, during low amino acid growth, by periodically loading the cells with high concentrations of amino acid. Figure 2 shows that the cells responded to alteration of external concentration of amino acid despite a high intracellular concentration, indicating that intracellular amino acid pool size was not a factor limiting control. As amino acids are probably evenly distributed between nucleus and cytoplasm[5], it was improbable that removal of the nucleus interfered with the initiation of the regulatory signal.

An alternative mechanism of substrate control of transport is by way of the rate of carrier activity per unit time. Enucleation itself caused a reduction in transport capacity; 0.5 h after enucleation, influx was decreased by 50% if expressed per cell.

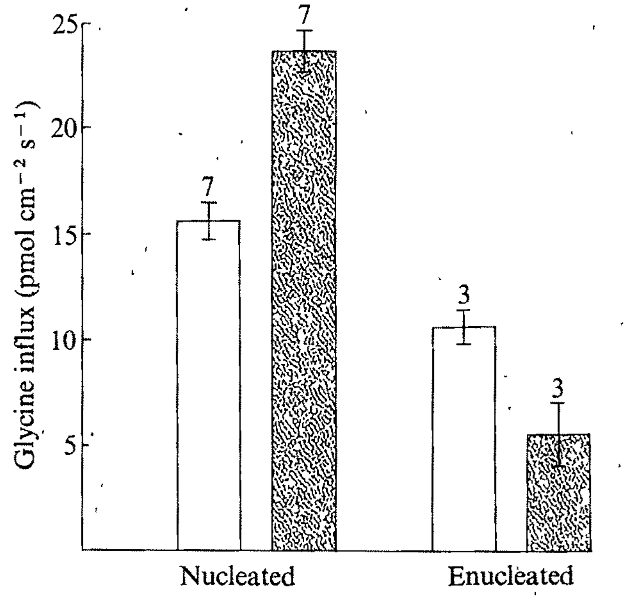

Fig. 1 The glycine-transport response of normal and enucleated HeLa cells to growth in low amino acid medium. HeLa cells were grown in normal Basal Medium Eagle (BME) on either glass coverslips (diameter 2.2 cm) or Falcon plastic Petri dishes (diameter 5 cm) to a final density of about 8 × 10^4 cells cm^{-2}. The cells were then incubated in growth medium containing 5 µg ml^{-1} cytochalasin B for 10 min and spun at 10,000 r.p.m. for 50 min at 37 °C. This procedure produced 60–80% enucleation and results were corrected for 100% enucleation. The cells were allowed to recover for 0.5–1 h in normal BME before further treatment. Cells were transferred to normal or low amino acid BME (stippled columns) for 10 h before glycine influx was measured. Low amino acid growth medium was prepared by omitting glutamine (2 mM), and glycine influx was taken as the initial uptake of 2-^{3}H-glycine in intracellular water, during a 5 min incubation at 37 °C (ref. 3). Different external concentrations of glycine were prepared by adding 2-^{3}H-glycine source to unlabelled glycine to give a specific activity of between 0.01 and 0.1 mCi mmol^{-1}. Radioactivity was determined by liquid scintillation counting of cell eluate, and quenching monitored by means of an external standard. Thin layer chromatography of cell extracts using *n*-butanol–water–acetic acid (12:5:3 by volume) solvent gave only a single peak which corresponded to glycine. Volume–surface area ratio of the cells was calculated assuming the cells were spherical. The number of observations are shown in each column. Means ± s.e. bars are given. Similar results were obtained in five experiments using cells grown on glass coverslips.

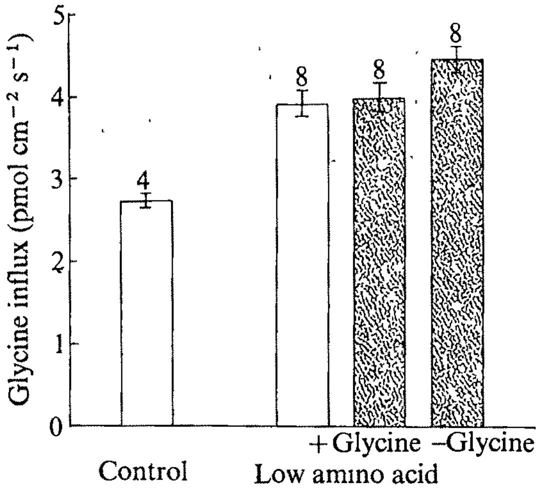

Fig. 2 The effect of altering intracellular amino acid concentration on the glycine transport-response of *Xenopus* cells to growth in low amino acid medium. Ordinate is glycine influx measured from 2 mM glycine* solution at 26 °C. Cells were placed into low amino acid growth medium at time zero. At intervals of 2 h thereafter some cells (stippled columns) were 'lysed'[6], refilled with either zero or 80 mM glycine and returned to the low amino acid growth medium. The response to extracellular amino acid concentration occurred whether intracellular concentration was high or low (Student's *t* test $P<0.001$). The number of observations are shown in each column. Values given are means ± s.e. bars.

It is probable that some cell membrane was lost during the enucleation procedure ('wrapped' around the nucleus). Loss of cell membrane was approximately 47%, a value sufficient to explain the loss of transport capacity in terms of carrier loss rather than carrier activity.

Incubation of enucleated cells in low amino acid BME caused a decrease rather than an increase in their glycine influx (Fig. 1). This may represent a secondary regulatory mechanism, an increase in the rate of catabolism of a labile carrier protein. This presupposes the continuous synthesis of protein directly involved in carrier-mediated active transport.

R.W. was a Leverhulme Scholar on leave from Department of Physiology, University of Berne. This work was supported by the MRC.

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Specificity of cellular retinol-binding protein for compounds with vitamin A activity

THE importance of vitamin A (retinol) for normal growth and development of vertebrates is well established[1] but its mechanism(s) of action, except in vision, remains obscure. Retinol can influence differentiation of epithelial cells[2] and is required for normal development of the testis and for maintenance of pregnancy[3], suggesting a regulatory role resembling that of certain hormones in some aspects of its activity. The action of many steroid hormones is apparently mediated through specific cellular binding proteins[4]. We have found that rat tissues[5,6] and human[5] and rabbit lungs[7] contain a protein able to bind all-*trans*-retinol, the most potent vitamin A compound, with high affinity and specificity. In all species studied so far, this protein has the same apparent size (molecular weight 14,000, sedimenting in the 2S region on sucrose gradients) and seems to carry retinol as a non-covalently bound ligand *in vivo*[6,7]. It has a lower molecular weight and a different fluorescence excitation spectrum[6] than that reported for the intensively studied retinol binding protein of serum[8], demonstrating that we are dealing with a different protein. We have purified this cellular binding protein 1,000-fold from rat testes[6] and 600-fold from rabbit lung[7] and report here its specificity in binding various compounds exhibiting vitamin A activity *in vivo*.

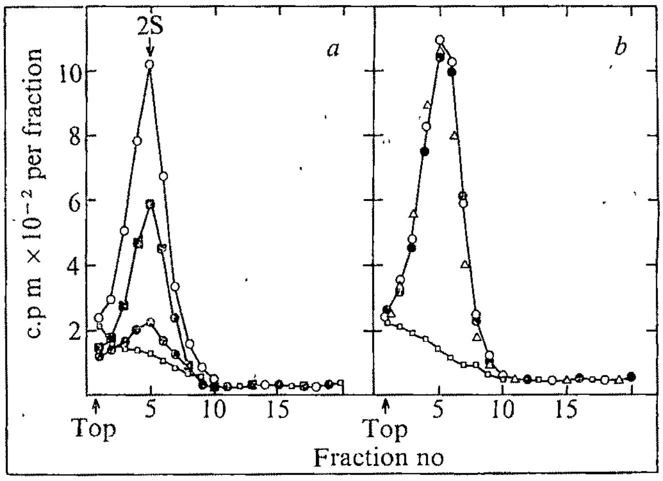


Fig. 1 Binding of all-*trans*-15-^{3}H-retinol (specific activity 1.25 Ci mmol^{-1}, New England Nuclear Corp.) to rat testis retinol-binding protein purified 1,000-fold. A sample (0.2 ml) of an 0.5-ml incubation mixture containing 10 μg of protein, 4×10^{-8} M all-*trans*-^{3}H-retinol with and without 8×10^{-6} M competitor, 0.05 M Tris-HCl, *p*H 7 6, was layered on a 5–20% sucrose gradient in 0.05 M Tris-HCl, *p*H 7.6 and centrifuged in a SW 50.1 rotor for 20h at 4 °C and 148,000*g*. Fractions of 0.25 ml were collected and radioactivity was determined. *a*· ○, ^{3}H-retinol only; ■, plus 9-*cis* retinol; ●, plus 13-*cis*-retinol; □, plus all-*trans*-retinol. *b*: ○, ^{3}H-retinol only; △, plus retinyl palmitate; ●, plus retinal; □, plus α-retinol.

Samples of the partially purified protein were incubated with radioactive all-*trans*-retinol in the absence and presence of excess unlabelled test compound and then subjected to sucrose gradient centrifugation. Retinol in aqueous solution adsorbed somewhat to glass and to the polyallomer centrifugation tubes and generally did not appear at the top of the gradient after centrifugation as would be expected for a non-adsorbed small molecule. Binding was indicated by a peak of radioactivity in the 2S region of the gradient. Gradients for some of the compounds tested are shown in Fig. 1. The results of all binding competition experiments are presented in Table 1 and expressed as percentage inhibition (percentage decrease) of binding of radioactive all-*trans*-retinol observed when the competitor was present in a 200-fold molar excess over the ^{3}H-retinol. The relatively large excess of competitor was used to detect compounds with low affinity for the binding protein. The actual numbers obtained are interpreted to indicate only the relative ability of the listed compounds to interact with the binding protein.

Of the *cis*-isomers tested, using the binding protein from both rat and rabbit, 13-*cis*-retinol was the most effective competitor although less effective than all-*trans*-retinol, while 9-*cis* and 9,13-di *cis*-retinol had significantly less but approximately equal abilities to compete, compared with all-*trans*-retinol. It is interesting that the ability of the *cis*-isomers to stimulate growth in the vitamin-A-deficient rat (last column, Table 1) followed the same pattern, with 13-*cis*-retinol the most effective, while 9-*cis*

Table 1 Percentage inhibition of binding of all-*trans*-^{3}H-retinol to protein by vitamin A active compounds

Compound*	Rat binding protein %	Rabbit binding protein %	*In vivo* activity (rat) (%)
All-*trans*-retinol	100	100	100
Cis-isomers			
13-*cis*-retinol	95	92	75 (ref.) 9)
9-*cis*-retinol	51	66	22 (ref. 9)
9,13-di *cis*-retinol	64	62	24 (ref. 9)
Ring isomers			
α-retinol	100	100	2 (ref. 10)
3-dehydroretinol (vitamin A_2)	100	100	40 (ref. 11)
C-15 modifications			
retinal	0	0	91 (ref. 10)
retinoic acid	0	0	100 (ref. 12)
retinyl acetate	0	0	100 (ref. 9)
retinyl palmitate	0	0	100 (ref. 13)

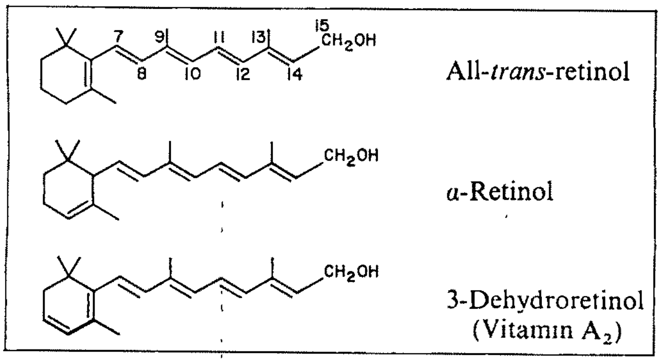
*Added in 200-fold molar excess over all-*trans*-^{3}H-retinol (8×10^{-8} M).

and 9,13-di *cis*-retinol had lower and approximately equal activities, compared with all-*trans*-retinol. These data suggest that the ability to interact with the cellular binding protein is one of the factors which determines whether a compound possesses vitamin A activity.

In contrast, both α-retinol and 3-dehydroretinol (Fig. 2) competed as well as all-*trans*-retinol in our binding assay, whereas their *in vivo* activities have been established to be 2% (ref. 10) and 40% (ref. 11), respectively, compared with all-*trans*-retinol. When administered to the rat, however, α-retinol is stored in the liver with high efficiency[10] and, at high doses, fully supports growth and development[14]. Goodman *et al.*[14] observed that α-retinol could not enter into the normal serum transport system and so delivery to target tissues is probably inefficient. Clamon *et al.*[15] have shown that when α-retinyl acetate is added directly to serum-free organ cultures of hamster trachea with keratinised squamous lesions induced by vitamin A deficiency, it is virtually as effective as all-*trans*-retinyl acetate in reversing the metaplasia. Apparently, the change in ring confirmation prevents the release of α-retinol from the liver to the serum transport system leading to a low *in vivo* activity, which does not necessarily reflect its potency in a target tissue or conflict with its demonstrated ability to interact with the tissue retinol binding protein (Fig. 1*b*). This sensitivity to ring conformation may also explain why 3-dehydroretinol exhibits less activity than all-*trans*-retinol *in vivo*[11].

Finally, the binding protein showed a stringent requirement for the alcohol function at C-15, as neither retinal, retinoic acid nor esters of the alcohol competed for binding (Fig. 1*b*). Since retinal can be reduced to retinol[16] *in vivo* and the esters hydrolysed to retinol[17], their biological activity may represent their conversion to all-*trans*-retinol, which is bound so effectively by the binding protein. Retinoic acid is not reduced to the alcohol *in vivo*[18] and although it reverses some of the lesions of vitamin A deficiency, it cannot prevent degeneration of the testes or maintain pregnancy in the rat[3]. It does not seem to act through the retinol-binding protein described here. We have found that several rat tissues contain a binding protein specific for retinoic acid and separable from that binding retinol[6]. This protein might be similar to that described in chick embryonic skin[19]. The action of retinoic acid may be mediated by its own binding protein.

Fig. 2 Chemical structures of all-*trans*-retinol, α-retinol and 3-dehydroretinol.



This work was supported by grants from the US Public Health Service. We thank Hoffmann-LaRoche for the compounds tested.

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Received February 3, revised March 14, 1975.

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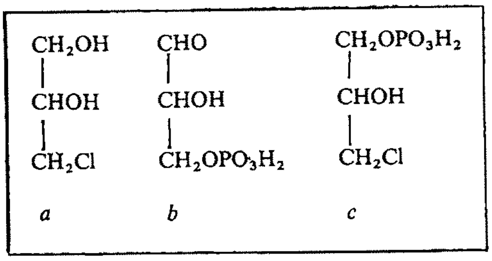
Identification of the biochemical lesion produced by α-chlorohydrin in spermatozoa

THE potential of α-chlorohydrin (3-chloro-1,2-propanediol; Fig. 1*a*) as an orally active reversible male antifertility agent has been demonstrated in the rat[1,2], ram[3] and boar[4]. Adverse side-effects, however, particularly in the monkey[5], have precluded human trials and for this reason, elucidation of the mechanism of action[6,7] of α-chlorohydrin is clearly important in order to develop less toxic yet more active antifertility analogues. Although high doses obstruct the lumen of the caput epididymis of the rat[2], the initial and primary contraceptive effect seems to be directly on spermatozoa within the cauda epididymis[8–11].

It has recently been shown in this laboratory that α-chlorohydrin is a potent inhibitor of glycolysis in ram spermatozoa both *in vitro* and *in vivo*. Thus, when washed ram spermatozoa (collected by electroejaculation) were incubated for 3 h with U-^{14}C-glucose (15 mM) or U-^{14}C-fructose (10 mM) in the presence of α-chlorohydrin (0.1 mM), no lactate accumulated, while oxygen uptake and the radioactivity recovered in the CO_2 were decreased to 50% of the control values. On the other hand, incubation of spermatozoa with U-^{14}C-pyruvate (5 mM) or U-^{14}C-lactate (5 mM) required α-chlorohydrin at a concentration of 10–100 mM to cause a 50% inhibition of oxygen uptake and oxidation of the substrates to CO_2. Inhibition of respiration caused by the oxidation of endogenous substrates, presumably lipid, was negligible until 100 mM α-chlorohydrin was added, when the oxygen consumption was decreased by only 20%. In another experiment the oxygen uptake of ram spermatozoal mid-pieces, which are essentially mitochondria[12], was monitored during incubation with either α-glycerophosphate (20 mM), succinate (20 mM) or pyruvate



$$\begin{array}{ccc} CH_2OH & CHO & CH_2OPO_3H_2 \\ | & | & | \\ CHOH & CHOH & CHOH \\ | & | & | \\ CH_2Cl & CH_2OPO_3H_2 & CH_2Cl \\ a & b & c \end{array}$$

Fig. 1 Structures of *a*, 3-chloro-1,2-propanediol; *b*, glyceraldehyde-3-phosphate; *c*, DL-1-chloro-1-deoxyglycerol-3-phosphate.

(20 mM)+malate (20 mM). The oxygen consumption, however, was not significantly affected by the addition of α-chlorohydrin at concentrations up to 100 mM.

From these results it is obvious that the glycolytic, rather than a subsequent oxidative pathway, is particularly vulnerable to α-chlorohydrin in spermatozoa. Positive identification of the inhibited site(s) was determined by measurement of the glycolytic intermediates following incubation of ram spermatozoa with fructose (15 mM) in the presence and absence of α-chlorohydrin (3 mM). It is clear from the 'cross-over' plot (Fig. 2) that the inhibited site is the formation of 3-phosphoglycerate (3 PGA) from glyceraldehyde-3-phosphate (GA3P; Fig. 1*b*). This step actually involves two enzymes, glyceraldehyde-3-phosphate dehydrogenase (GA3Pdh) and phosphoglycerate kinase. It is unlikely that the latter enzyme is inhibited because if it were, the intermediate compound, 1,3-diphosphoglycerate, would accumulate. This intermediate is extremely unstable and rapidly breaks down to 3PGA. Absence of the accumulation of 3PGA suggests that GA3Pdh is inhibited by α-chlorohydrin.

We investigated the direct effect of α-chlorohydrin on spermatozoal GA3Pdh. Enzyme extracts were prepared from washed ram spermatozoa by sonication for 5 min in sucrose (0.25 M) containing EDTA (10^{-3} M) and β-mercaptoethanol (10^{-3} M). Inclusion of α-chlorohydrin at concentrations as high as 300 mM in the reaction cuvette, however, had no effect on the activity of GA3Pdh or any of the other glycolytic enzymes assayed including; hexokinase, phosphofructokinase (PFK), aldolase, triosephosphate isomerase (TPI), phosphoglycerate kinase, pyruvate kinase or lactate dehydrogenase (LDH). From these results we conclude that α-chlorohydrin itself does not inhibit GA3Pdh directly.

The possibility of an indirect action due to a metabolite of α-chlorohydrin was tested by incubating washed spermatozoa for 3 h with fructose (15 mM) in the presence of a range of concentrations of the compound (0.03–300 mM). After incubation, the spermatozoa were separated by centrifugation and enzyme extracts of the cells prepared by sonication as described above. The results (Table 1*a*) showed that both GA3Pdh and TPI were severely inhibited at all concentrations of α-chlorohydrin, although the effect was greater on GA3Pdh than TPI. No other glycolytic enzymes were significantly affected except PFK and LDH which showed some inhibition at 300 mM α-chlorohydrin (not shown).

Similar studies have been carried out on ejaculated spermatozoa collected from rams which were injected for 4 d with α-chlorohydrin (25 mg per kg body weight daily). GA3Pdh activity was completely lost within 24 h of the first injection (Table 1*b*) and the activities of both TPI and aldolase were also severely inhibited compared with the preinjection levels. During the postinjection period the activities of these enzymes slowly returned to the preinjection levels and by 26 d this was essentially complete. The motility of the ejaculated spermatozoa was also monitored and always found to parallel the GA3Pdh activity.

The evidence, both *in vitro* and *in vivo*, is conclusive that aldolase, TPI and particularly GA3Pdh are severely inhibited by α-chlorohydrin, so that the flux of carbon through glycolysis ceases. Sufficient energy (from carbohydrate) is therefore not available to ejaculated spermatozoa for essential requirements, such as motility in the female tract and this may render them infertile.

Very recent experiments with the C_1 phosphorylated derivative of α-chlorohydrin (DL-1-chloro-1-deoxyglycerol-3-phosphate; Fig. 1*c*) suggest that this compound may be a

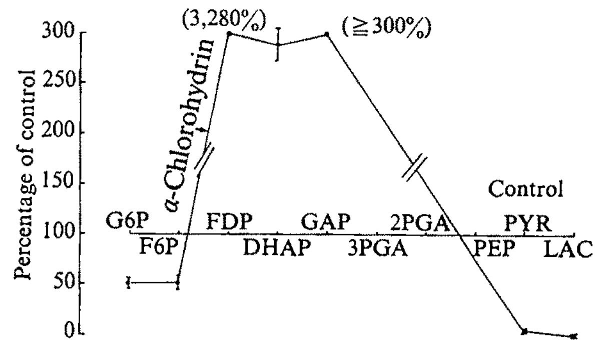


Fig. 2 'Cross-over' plot of the glycolytic intermediates of ram spermatozoa after incubation with fructose (15 mM) and α-chlorohydrin (3 mM). The glycolytic intermediates from the α-chlorohydrin treated spermatozoa are expressed as a percentage of the control values, which are arbitrarily called 100%. The phosphoglycerates and phosphoenol pyruvate were too low to be detected in both control and experimental spermatozoal extracts by the fluorometric methods used[13].

Table 1 Effect of α-chlorohydrin *in vitro* and *in vivo* on the activity of some glycolytic enzymes of ram spermatozoa

a After incubation of spermatozoa with fructose (15 mM) and different concentrations of α-chlorohydrin

Enzymes	Enzyme activity* α-chlorohydrin (mM)†					
	None	300	30	3	0.3	0.03
Triosephosphate isomerase	4.40	0.18	0.5	0.84	1.69	1.91
Glyceraldehyde-3-phosphate dehydrogenase	0.45	0.00	0.00	0.03	0.11	0.17

b Before, during and after injection of rams with α-chlorohydrin

Enzymes	Enzyme activity*						
	Preinjection	During injection		Post injection			
		Day 4	Day 4	Day 4	Day 11	Day 18	Day 26
	(5)	(3)	(1)	(4)	(4)	(5)	(3)
Aldolase	0.41±0.02	0.25±0.05	0.13	0.14±0.07	0.30±0.09	0.37±0.07	0.44±0.03
Triosephosphate isomerase	5.58±0.56	0.72±0.28	0.45	0.20±0.02	2.70±1.02	3.45±0.67	4.28±0.25
Glyceraldehyde-3-phosphate dehydrogenase	0.40±0.01	ND	ND	0.002±0.001	0.23±0.09	0.29±0.08	0.38±0.02

*Measured as μmol min^{-1} per 10^9 spermatozoa at 25 °C.

†Values are means of duplicate observations from a pool of 3 ram ejaculates. The differences between the duplicate observations are within 5%.

Values are means ± s.e.m. Five animals were involved in this experiment but all five ejaculates were not always available, the number of ejaculates used for each test is therefore shown in parentheses.

ND, Not detectable. Aldolase and GA3Pdh were assayed according to Wu and Racker[14] and TPI by the method of Beisenherz[15].

metabolic product of α-chlorohydrin which directly affects GA3Pdh, TPI and aldolase. Addition of DL-1-chloro-1-deoxyglycerol-3-phosphate (0.1 mM) severely inhibited the activity of GA3Pdh (53%) in the reaction cuvette containing spermatozoal sonicate. One hundred times that concentration of inhibitor was required for significant inhibition of TPI and aldolase. We suggest that α-chlorohydrin is phosphorylated on entry into the spermatozoa. The phosphorylated compound may then act as a competitive inhibitor of GA3Pdh because of its structural similarity to the normal substrate of the enzyme, glyceraldehyde-3-phosphate. Further investigations of this hypothesis are presently under way.

We thank Professor C. W. Emmens for his interest and advice and the Upjohn Company of Kalamazoo, Michigan, for the gift of α-chlorohydrin. The investigation has received financial support from the World Health Organization.

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Localisation of plutonium in mouse testes

THE plutonium isotope ^{239}Pu is a known carcinogen but its ability to produce genetic damage has not been so well investigated. The radionuclide is found in gonads after intravenous injection into humans[1] and after administration to animals by various routes[2]. Retention of ^{239}Pu in the gonads is prolonged and the genetic effects there have been estimated on the basis of average dose in the gonads[3]. Inhomogeneity of distribution of plutonium within gonads has, however, been noted[4] giving rise to the possibility that some cells may receive a greater radiation dose than others. No investigations of the effects of this inhomogeneity upon dose-rate to the cells involved in gametogenesis have been reported. Here we report briefly that a non-uniform distribution of ^{239}Pu in the testis results in increased radiation of the spermatogonial stem-cells.

Groups of 12-week-old male CBA mice were killed 4 or 12 weeks after intravenous injection of ^{239}Pu citrate. The plutonium in each right testis was measured radiochemically (Table 1). Autoradiographs prepared from 6-μm sections of frozen left testes showed that some 90% of the plutonium seemed to be deposited within the intertubular spaces and in the peritubular tissue immediately surrounding them.

Table 1 Radiochemical analysis of the right testis of CBA mice following intravenous injection of ^{239}Pu in 1% trisodium citrate solution

Weeks after injection	4	12
Number of testes	5	6
Testis mass (mg, mean ± s.e.)	73±1	67±4
Plutonium (pCi, mean ± s.e.)	20±3	23±2
Plutonium (pCi g^{-1}, mean ± s.e.)	280±45	350±38
Average dose rate (rad d^{-1}, mean ± s.e.)	0.072±0.012	0.092±0.010

A mean activity of 88 nCi ^{239}Pu was injected into each mouse; 3.2 μCi per kg body mass.

A total of 12,000 α tracks were counted in more than 700 randomly selected fields from 58 sections.

The α tracks could be categorised into: those which lay entirely above the intertubular spaces (47%); those which lay above the peritubular membrane or above the adjoining 10-μm layer of cells containing the spermatogonial stem cells (42%); and those which lay entirely within the peritubular membranes but excluding those in group 2 (11%).

On the assumption that the testis consists of a close-packed series of cylinders of uniform diameter (experimentally determined mean value =206 μm) it was calculated that the 10-μm shell of tissue surrounding each tubule and containing the spermatogonial stem-cells, constituted 17% of the whole testis mass. It was then assumed that the spermatogonial stem-cells were randomly distributed in these 10-μm peritubular shells and a dose inhomogeneity factor N (ref. 5) was calculated:

$$N = \frac{\text{average dose to spermatogonial stem-cells}}{\text{average dose to whole testis}}$$

$$= \frac{\text{fraction of all } \alpha \text{ tracks in group 2}}{\text{fraction of testis mass in 10-}\mu\text{m peritubular shells}}$$

$$= \frac{0.42}{0.17} = 2.5$$

Therefore, from the data given in Table 1, dose rates to spermatogonial stem-cells in our experiment were 0.18 and 0.23 rad d^{-1} at 4 and 12 weeks respectively. A value $N=2$ was also calculated directly from the model system assuming random distribution of ^{239}Pu in the intertubular spaces and taking into account the transfer of energy along the path of each α particle (M. C. Thorne, personal communication).

Our results show that for mice the radiation dose rate to the spermatogonial stem-cells is greater by a factor of 2–2.5 than the average for the testis as a whole calculated directly from the total amount of ^{239}Pu deposited in the gland. This implies that the genetic effects may be proportionately greater. The validity of the factor N as calculated and the biological effectiveness of α particles relative to other radiations are matters now undergoing cytological investigation (A. G. Searle, personal communication). It would also be important to establish comparable values for ovaries. Much more work is required to decide on appropriate values for men.

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Modification of drug biotransformation by vitamin C in man

VITAMIN C (ascorbic acid) is taken by many individuals in very large doses and there has been some concern about possible adverse effects of such regimens. It has been found that vitamin C is metabolised in part to ascorbic acid sulphate in man[1]. Sulphate formation is also an important pathway for the biotransformation of phenolic drugs[2]. This pathway is, however, of limited capacity in man[3] (and in many species of animals) and is therefore subject to saturation and competitive inhibition. Such mutual inhibition by two or more substrates is caused by the limited availability of sulphate and can be overcome by concomitant administration of sulphate or a sulphate donor[3]. Studies in man have demonstrated competitive inhibition of sulphate formation between acetaminophen and salicylamide, two widely used analgesics and antipyretics, and the prevention of this inhibition by concomitant administration of the sulphate donor L-cysteine[4]. We now report the results of a study which shows that concomitant administration of vitamin C and salicylamide causes a decrease in the conversion of the latter to salicylamide sulphate.

The results of the study are summarised in Table 1. Recovery of total salicylamide (that is, the sum of all urinary excretion products of the drug) was essentially quantitative (95% of the dose) and was not affected by vitamin C. The fraction of the dose excreted as salicylamide sulphate was significantly decreased and that excreted as salicylamide glucuronide was increased by concomitant administration of vitamin C. Eight of the ten subjects showed this decrease in salicylamide sulphate output; the effect was particularly pronounced in subjects C, H, I and L. There was a corresponding, statistically significant change in the average maximum excretion rates of the metabolites: 0.562 and 0.513 mg salicylamide equivalent per min for the sulphate in the control and vitamin C experiments, respectively ($P < 0.02$ by paired t test), and 0.715 and 0.824 mg salicylamide equivalent per min for the glucuronide in the control and vitamin C experiments, respectively ($P < 0.005$ by paired t test). The vitamin had no statistically significant effect on the pH and flow rate of urine.

Previous studies have shown that the extent to which salicylamide is converted to salicylamide sulphate in man is decreased with increasing dose, increased by administration of a sulphate donor and increased by decreasing the rate of absorption of the drug[3]. Vitamin C may decrease the rate of absorption of salicylamide because the vitamin inhibits gastric emptying in man[5]. Thus, the vitamin may cause a decrease in salicylamide sulphate formation by one mechanism and an increase by another mechanism, so that a typical dose–response relationship for the effect of vitamin C on sulphate formation should not be expected. It is likely that there is an optimum dose of vitamin C for maximum reduction of salicylamide sulphate formation, a dose at which the inhibitory effect of the vitamin on salicylamide absorption is relatively less pronounced than its effect on sulphate formation. This optimum dose probably differs between individuals and may differ also as a function of dosage form (solution, tablets, and so on) and drug (that is, drugs other than salicylamide which are also subject to sulphate formation). It is also likely that individuals differ in their capacity for vitamin C absorption (the vitamin is absorbed by a specialised saturable process[6]) and in the rate at which endogenous sulphate is available for conjugation with a substrate such as salicylamide. All of these variables may account for the interindividual variations in the effect of vitamin C on the formation of salicylamide sulphate as found in this study.

A number of drugs are extensively conjugated with sulphate (and thereby inactivated) in the first pass through the intestinal tissues and liver following oral administration. One such drug is isoprenaline (isoproterenol) which is used widely for the treatment of asthma and certain cardiac diseases. The recommended

Table 1 Effect of vitamin C* on the biotransformation of salicylamide† in man

Subject	Age (yr)	Total recovery in urine (% dose)		Salicylamide sulphate (% dose)		Salicylamide glucuronide (% dose)	
		Control	Vitamin C	Control	Vitamin C	Control	Vitamin C
A	25	93.4	101.1	31.6	25.9	54.4	59.8
C	25	91.8	92.7	35.0	25.7	52.1	60.1
D	25	94.7	95.7	29.3	29.9	55.9	58.8
G	47	91.2	92.7	46.5	41.5	38.5	46.5
H	26	97.9	96.2	29.7	19.7	58.8	69.6
I	27	94.5	94.8	39.9	28.9	46.3	53.7
L	39	98.0	98.1	35.8	25.4	53.7	64.5
O	32	95.3	94.4	48.9	42.9	40.0	43.8
S	32	96.6	92.7	35.7	31.1	57.2	59.2
Y	29	96.1	96.3	35.7	38.7	56.3	53.6
Mean		94.9	95.5	36.8	31.0	51.3	57.0
S.d.		2.3	2.7	6.6	7.7	7.2	7.8
Statistical significance of difference from control‡		NS		$P < 0.005$		$P < 0.005$	

Ten healthy male volunteers 25–47 yr old, capable by education and experience to give their informed consent (research assistants and associates), participated in the study. They were instructed not to take any drugs or vitamins for at least one week before and during the study. They fasted overnight, emptied their bladder in the morning and took 100 ml of water or 2 g of vitamin C in 100 ml water. One hour later, they collected their urine and took 150 mg salicylamide alone or with 1 g vitamin C, both dissolved in 100 ml water. No food or drink other than water was permitted for the following 2 h. Urine was collected at 0.5, 1, 1.5 and 2 h after salicylamide administration and at convenient times thereafter for a total of 24 h, each time followed by 100 ml water to assure adequate urine flow. Five of the subjects took salicylamide with vitamin C, whereas the other five took salicylamide only. At least 1 week later, the former took salicylamide alone, whereas the latter took it with vitamin C. The urine samples were analysed for total salicylamide and its metabolites (salicylamide sulphate, salicylamide glucuronide and gentisamide) as described previously[3]. Blank values obtained from the 1 h urine sample collected before salicylamide administration were used to correct the analytical results; blank values from urine obtained after administration of the initial 2-g dose of vitamin C did not differ from those obtained without prior ingestion of the vitamin. Several urine samples obtained after administration of salicylamide alone were diluted with an equal volume of urine either from unmedicated subjects or from subjects taking vitamin C; there were no differences in analytical results, showing that the vitamin and its metabolites did not interfere with the analysis of salicylamide and its metabolites. Some urine samples obtained after ingestion of salicylamide alone and after ingestion of salicylamide with vitamin C were analysed immediately after collection and again after 10 d of storage in a refrigerator. These analyses yielded essentially identical results, thereby ruling out a possible effect of ascorbic acid or its metabolites on the stability of the salicylamide metabolites.

*2 g taken 1 h before salicylamide and 1 g taken concomitantly.

†150 mg salicylamide dissolved in 100 ml water.

‡Paired t test.

oral dose of this drug is about 100–1,000 times larger than its intravenous dose[7], reflecting largely its extensive inactivation by biotransformation during absorption. It has been predicted[8] and demonstrated in dogs[9] that salicylamide can markedly inhibit this inactivation. Following introduction of isoprenaline into the intestinal lumen of anaesthetised dogs, only 3.6% of the total amount absorbed was unmetabolised drug; 68% was isoprenaline sulphate. When isoprenaline was given with salicylamide, 73.7% of the absorbed amount was unmetabolised isoprenaline and only 3.3% was in the form of the sulphate conjugate, on the average. Similar effects may occur when vitamin C is taken with drugs which ordinarily undergo extensive conjugation with sulphate during their initial passage across the intestinal mucosa and through the liver. This could result in a significant potentiation in the pharmacological activity of drugs so affected.

Supported in part by a grant from the National Institute of General Medical Sciences, NIH, Bethesda, Maryland.

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Received February 17; accepted March 12, 1975

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Tryptophan pyrrolase activity regulation in *Drosophila:* role of an isoacceptor tRNA unsettled

TRYPTOPHAN pyrrolase is a key enzyme in the biosynthetic pathway leading to the ommochromes[1]. In wild-type flies of *D. melanogaster* or *D. hydei*, tryptophan is converted by this enzyme into *N*-formyl kynurenine, which is hydrolysed to formic acid and kynurenine. Further metabolism of kynurenine results in the deposition of the yellow-brown pigments in the eyes of these insects. Tryptophan pyrrolase activity, however, is lacking or greatly reduced in freshly prepared homogenates of living imagines of the vermilion mutants of *D. melanogaster*[1,2,3] or *D. hydei* (Table 1). Finding a stimulation of tryptophan pyrrolase activity in homogenates of *D. melanogaster* vermilion flies by RNase T_1 treatment and an inhibition of this activity by wild-type tRNA preparations or by an isoaccepting tyrosine tRNA ($tRNA_2^{Tyr}$) led Jacobson[4,5] to suggest the implication of this specific isoaccepting tRNA in the regulation of tryptophan pyrrolase activity and in the mechanism of genetic suppression of the vermilion mutant. Because of the importance of these conclusions and their bearing on the general understanding of the basic molecular mechanisms of enzyme regulation and suppression in eukaryotes, we reinvestigated the entire system including the assay procedure.

Tryptophan pyrrolase activity is assayed on the basis of the amount of kynurenine produced from tryptophan in homogenates of the respective insect tissues. This assay, however, is usually hampered by microbial contamination and the lack of a good technique for the direct determination of the kynurenine produced in the assay mixture. Avoiding the unreliable and troublesome diazotation reaction of Bratton and Marshall[6], we identified kynurenine by its R_f value and typical light-blue fluorescence after thin-layer chromatography on cellulose powder, and quantitated kynurenine on the basis of its extinction at 360 nm. To eliminate microbial contamination, penicillin and streptomycin were included in the assay mixture.

All our attempts failed to induce tryptophan pyrrolase activity in freshly prepared homogenates of *D. melanogaster* or *D. hydei* vermilion flies by RNase T_1 or RNase A treatment (Table 1) following exactly the procedure published[4]. This failure cannot be attributed to either lack of RNase activity or a difference in strain: the vermilion mutant was suppressable with the *D. melanogaster* vermilion suppressor *su*(s)[2], and RNase activity stays very high for several hours in our assay system.

Tryptophan pyrrolase activity can be stimulated to almost wild-type activity levels when homogenising vermilion flies in the presence of 0.02 M EDTA, a standard ingredient in RNase assay mixtures. To prove that the substance produced in the EDTA activated vermilion mutants is kynurenine, 2-^{14}C-tryptophan was added to our standard tryptophan pyrrolase assay mixture. The resulting product was purified to constant specific radioactivity, and the identity of this radioactive compound with L-kynurenine was demonstrated by thin layer chromatography on cellulose powder in three different solvent systems. In the presence of EDTA, tryptophan pyrrolase activity is generally stimulated in homogenates of wild-type and vermilion mutant strains of *D. melanogaster* as well as *D. hydei*, independent of the developmental stage of the respective organisms (Table 1).

The tryptophan pyrrolase activity stimulated by EDTA in the vermilion homogenates could not be blocked by tRNA preparations of the respective wild strains. The EDTA stimulated tryptophan pyrrolase activity of *D. melanogaster* vermilion imagines was separated from the EDTA by passing the homogenate through a Sephadex G-75 column. This activity could also not be blocked by the addition of a purified tRNA preparation of wild-type *D. melanogaster* imagines (Table 1).

Table 1 shows an additivity of the normal and EDTA stimulated tryptophan pyrrolase activity, from which it may be

Table 1

Organism and treatment*	Kynurenine (μg, average produced by 100 mg of fresh tissue) Without EDTA	With EDTA	EDTA-activated, tRNA added
D. melanogaster wild strain, imagines	46	70	—
D. melanogaster vermilion, imagines	ND	32	18
D. melanogaster vermilion, imagines, RNase T_1	ND	36	—
D. melanogaster vermilion, imagines, RNase A	ND	35	—
D. melanogaster vermilion, IIIrd instar larvae	ND	26	—
D. melanogaster vermilion, imagines, EDTA removed	—	—	14
D. hydei wild strain, imagines	26	36	—
D. hydei wild strain, IIIrd instar larvae	10	28	—
D. hydei vermilion, imagines	ND	14	—

*Living organisms (500 mg) were homogenised in 5.5 ml Tris-HCl (0.01 M, *p*H 7.5) supplemented with 3 mg mercaptoethanol, 4 mg ascorbate, 4.3 mg streptomycin and 2.15 mg penicillin Following centrifugation, either 1 ml tryptophan solution (5 mg ml^{-1} homogenisation medium) or 1 ml homogenisation medium was added to 1.5 ml supernatant. Incubation was for 3 h at 37 °C Ethanol was added to a final concentration of 70% and kynurenine production was determined as in the text. Reproducibility of kynurenine determination in duplicate experiments was better than 95%. For tryptophan pyrrolase activity stimulation the homogenisation medium was made 0.02 M in EDTA. Purified tRNA of *D. melanogaster* wild strain imagines (10 μg ml^{-1}), RNase T_1 (up to 1,500 U) and RNase A (4 units) were present in the reaction mixture where indicated.

ND, not detectable.

inferred that the tryptophan pyrrolase activity stimulated by EDTA possibly stems from an enzyme (perhaps a peroxidase) other than the original tryptophan pyrrolase molecule.

On the basis of our results it seems that the elegant idea of enzyme activity modulation in eukaryotes by varying proportions of specific isoacceptor tRNA molecules[4,5,7] still needs substantiation. In addition, the molecular basis of the mechanism of genetic suppression in eukaryotes[7,8] needs further elucidation.

We thank B. Chaillié and R. Klosson for assistance. This investigation was supported by the Deutsche Forschungsgemeinschaft.

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Received January 29; accepted March 19, 1975.

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Effects of cordycepin and cordycepintriphosphate on polyadenylic and ribonucleic acid-synthesising enzymes from eukaryotes

GENETIC information encoded in the nuclear DNA of a eukaryotic cell is selectively transcribed into an array of nuclear RNA molecules heterogeneous in size, base sequence and half-life. The α-amanitin-sensitive, DNA-dependent RNA polymerase isolated from nucleoplasm is thought to catalyse the transcription of heterogeneous nuclear RNAs (HnRNA). While in the nucleus, the length of HnRNAs is modified in two ways: first, HnRNAs are lengthened by addition of a poly(A) sequence. The ATP–polynucleotidylexotransferase isolated and purified from several eukaryotic tissues is presumed to catalyse the sequential addition of AMP moieties to the 3′ terminus of the HnRNA. Secondly, HnRNAs bearing poly(A) tails are thought to be shortened by cleavage of a portion of the nucleotide sequence at the 5′ terminus and the remainder transported to the cytoplasm. After association with ribosomal subunits, the processed transcripts form polysomes and function as mRNAs in the translation of specific polypeptides. Inferences as to the processing of HnRNA to give rise to mRNA are drawn from experiments using cordycepin (3′-deoxyadenosine) as an inhibitor of RNA synthesis[1-2]. Interpretation of many of these experiments rests on the assumption that the cordycepin selectively inhibits the addition of polyadenylic acid to HnRNA[4-6]. On the other hand, Klenow[7] has shown that cordycepin is phosphorylated to 3′-dATP by Erlich ascites tumour cells, and using a bacterial DNA-dependent RNA polymerase, Shigeura and Gordon[8] demonstrated inhibition of *in vitro* RNA synthesis by 3′dATP. They postulated that the mechanism of inhibition involved incorporation of the 3′-dATP into the growing chain of RNA with subsequent failure to provide a 3′-hydroxyl for the next incoming nucleosidetriphosphate to terminate elongation prematurely.

To directly determine if the mode of cordycepin inhibition of mRNA synthesis was on poly(A) addition to HnRNA or on the synthesis of HnRNA *per se* we tested the effect of cordycepin and 3′-dATP on a eukaryotic α-amanitin-sensitive, RNA polymerase and on two eukaryotic ATP–polynucleotidylexotransferases. The RNA polymerase was specifically inhibited by the 3′-dATP. The poly(A)-synthesising enzymes were no more sensitive to 3′-dATP than to 2′-dATP (the naturally occurring DNA precursor). All activities were refractory to low levels of cordycepin.

Table 1 Effect of cordycepin and 3′-dATP on poly(A) synthesis by HeLa ATP–polynucleotidylexotransferase

Drug	Concentration (mM)	Specific activity (nmol AMP mg^{-1})	Inhibition (%)
None	0	466	0
Cordycepin	3.6	277	41
Cordycepin	15.4	185	60
2′-d Adenosine	3.6	144	69
2′-d Adenosine	15.4	160	66
3′-dATP	0.22	356	24
3′-dATP	0.44	188	60
2′-dATP	0.22	331	29
2′-dATP	0.44	208	56

ATP–polynucleotidylexotransferase was prepared from HeLa S_3 cells by the method of Mans and Stein[14]. After step-wise elution from DEAE–cellulose the enzyme was purified further on a 2–20% glycerol gradient in 2.5 mM Tris (*p*H 8), 1 mM 2-mercaptoethanol and 100 mM $(NH_4)_2SO_4$ at 27,000 r.p.m. in an SW27 rotor at 4 °C for 68 h. Poly(A)-synthesising activity was assayed as described previously[15] with 1.7 A_{260} ml^{-1} *E. coli* tRNA as primer and the indicated drug.

To test directly the inferred inhibition of poly(A) synthesis by cordycepin and 3′-dATP we examined their effects on poly(A) synthesis by HeLa ATP–polynucleotidylexotransferase[14]. Cordycepin, at 15 times the level of substrate, inhibited the poly(A)-synthesising activity by only 60% (Table 1) and was no more potent than 2′-deoxyadenosine (2′-dA). The phosphorylated derivative, 3′-dATP, inhibited the HeLa exotransferase by only 24% at 0.22 mM and was no more potent than 2′-dATP. Therefore, *in vitro* poly(A) synthesis was insensitive to cordycepin and no more sensitive to 3′-dATP than to the naturally occurring DNA precursor, 2′-dATP.

The phosphorylated derivative, 3′-dATP, specifically inhibited the eukaryotic RNA polymerase to the same extent and with the same specificity as seen with the bacterial RNA polymerase (Fig. 1). Note that addition of 3′-dATP during the course of the reaction immediately halted AMP incorporation (Fig. 1*b*), consistent with the termination of elongating chains by the incorporation of 3′-dATP. The sensitivity of the eukaryotic DNA-dependent RNA polymerase to cordycepin was as slight and non-specific as that of the eukaryotic poly(A)-synthesising activity; no inhibition was observed with either 13.4 mM 3′-dA or 2′-dA.

The lack of specificity of inhibition of poly(A) synthesis in contrast to the strict specificity of inhibition of RNA synthesis by 3′-dATP is explicable. Poly(A) addition to an RNA primer and RNA synthesis on a DNA template proceed by different mechanisms of nucleotide incorporation. In poly(A) synthesis the RNA provides a 3′-hydroxyl terminus for its elongation as poly(A). Substrate specificity of the exotransferase is restricted to ATP and is conferred by the protein alone. In the RNA

Table 2 Effect of 3′-dATP on eukaryotic RNA polymerase utilisation of poly(dAT)

System	Inhibition of incorporation from 8-^{14}C-ATP (%)	Inhibition of incorporation from 2-^{14}C-UTP (%)
Complete less UTP	97	—
Complete less ATP	—	86
Complete plus 3′-dATP (0 11 mM)	95	96
Complete plus 2′-dATP (0 33 mM)	—	3

The complete reaction mixture was as described by Mans[18] except 0.52 A_{260} ml^{-1} poly(dAT) was substituted for DNA, and CTP and GTP were omitted.

Table 3 Effect of cordycepin and 3′-dATP on poly(A) synthesis by maize ATP–polynucleotidylexotransferase on several primers

Primer	Inhibition (%) of ^{14}C-AMP incorporation with			
	3′-dA(15.4 mM)	2′-dA(15.4 mM)	3′-dATP(0.22 mM)	2′-dATP(0.22 mM)
tRNA	48	36	69	49
Heated DNA	—	—	43	48
Poly(dT)^{10}	77	71	65	74
Poly(dT)	—	—	71	79

ATP–polynucleotidylexotransferase (fraction 5) was prepared from maize seedlings and assayed as described by Mans and Huff[15] except 0.44 A_{260} ml^{-1} of indicated primer and drug were added. The specific activity (nmol AMP incorporated per mg enzyme protein in 90 min at 30 °C) was 2,630, 3,050, 1,730 and 750 for the tRNA, DNA, poly(dT)$_{10}$ and poly(dT)-primed reactions, respectively.

polymerase reaction the DNA template serves to hydrogen bond each of the four nucleosidetriphosphates in proper sequence before their polymerisation by the enzyme. Substrate specificity of the RNA polymerase is extended to several ribonucleotides and is conferred by both the template and the enzyme. Both 2′-dATP and 3′-dATP have the adenine moiety required for exotransferase substrate specificity and compete effectively with ATP in binding to the enzyme. Although both 2′-dATP and 3′-dATP compete with ATP in hydrogen-bonding to the template only, the 3′-dATP has the 2′-hydroxyl in common with the natural substrates utilised by the RNA polymerase. Therefore, only 3′-dATP is incorporated into the RNA chain to subsequently terminate elongation.

Use of a deoxyoligomer as a template by the eukaryotic RNA polymerase was demonstrated by the requirement of ATP for ^{14}C-UMP incorporation and UTP for ^{14}C-AMP incorporation in the presence of the alternating copolymer, poly(dAT) (Table 2). Inhibition of ^{14}C-UMP as well as ^{14}C-AMP incorporation by 3′-dATP but not by 2′-dATP is again consistent with the known substrate specificity of the enzyme (for 2′ hydroxyl) mediated by base pairing (A–T) with the template. The homopolymer, poly(dT), also serves as a template for AMP incorporation that is sensitive to 3′-dATP but not to 2′-dATP (data not shown). Incorporation with either polymer is refractory to actinomycin D at 10 µg ml^{-1} anticipated with templates lacking guanosine residues.

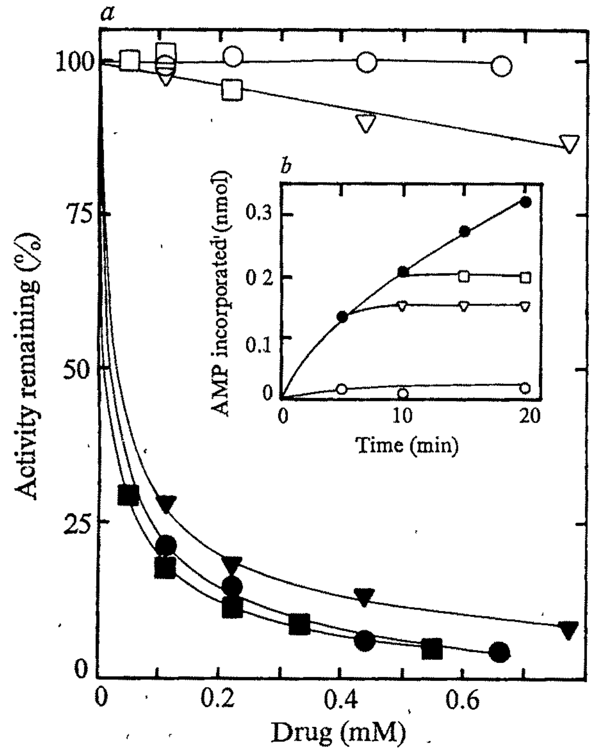

Fig. 1 *In vitro* effects of 3′-dATP on RNA synthesis. RNA polymerase (fraction 4) was prepared from *E. coli* K-12 and assayed on heated calf thymus DNA at 37 °C by the procedure of Burgess[17] (□, ■). RNA polymerase II (fraction 4) was prepared from maize seedlings and assayed on heated calf thymus DNA at 30 °C as described by Mans[18] with either 8-^{14}C-ATP (○, ●) or 2-^{14}C-UTP (▽, ▼) as labelled substrates. Either 3′-dATP (closed symbols) or 2′-dATP (open symbols) was added before incubation Nucleotide incorporated was determined as acid-insoluble radioactivity on filter paper disks[18]. No activity was detected on omission of enzyme or DNA or on addition of actinomycin D (10 µg ml^{-1}) to either enzyme assay or addition of α-amanitin (50 µg ml^{-1}) to the maize enzyme. The insert shows the rate of ^{14}C-AMP incorporation by the maize enzyme on addition of 3′-dATP (0.22 mM) after 0.5 min (○), 5.5 min (▽), 10.5 min (□) and no addition (●) incubation at 30 °C. Without drug the *E. coli* RNA polymerase incorporated 60 nmol AMP per mg protein in 10 min at 37 °C and the maize RNA polymerase incorporated 30 nmol AMP per mg protein in 10 min at 30 °C. HeLa S_3 cells were grown exponentially in suspension culture at 37 °C in Eagle's MEM supplemented with 3.5% each of calf and foetal calf serum[9]. At 5×10^6 cells ml^{-1}, 1 l of the cell culture was centrifuged (200*g* for 15 min at 37 °C). The packed cells were resuspended in 100 ml of the above medium containing 0.5 mg ml^{-1} cordycepin at 37 °C and incubated with stirring at 37 °C for 1 h. The 3′-dATP was isolated from the acid-soluble fraction by modification of the procedures of Klenow[7] and Shigeura and Gordon[8]. The cells were harvested by centrifugation at 0 °C and immediately resuspended in 3 ml 5% $HClO_4$ at 0 °C. After centrifugation the pellet was re-extracted with 5% $HClO_4$ at 0 °C and the combined supernantant fluids adjusted to *p*H 6 with M KOH. To oxidise the ribonucleotides, 0.56 ml 0.25 M $NaIO_4$ was added to 9.8 ml supernatant fluid at 23 °C for 1 h (ref. 10). After cooling the mixture to 0 °C, 0.1 ml cyclohexylamine was added and the *p*H adjusted to 6.4 with acetic acid. The mixture was adsorbed to activated charcoal and the deoxynucleotides eluted with ethanol–pyridine–H_2O (50:5:45). The ethanol and pyridine were removed by evaporation and the remaining aqueous phase (3.7 ml) was applied to a Dowex 1-formate column (0.6×23 cm). The nucleotides were eluted with a 440 ml linear gradient of 0–2 M NH_4COOH (*p*H 4.5) at 11 ml h^{-1}. Nucleosidetriphosphates, lesser amounts of mono- and diphosphonucleotides were eluted in discrete peaks from the column with the NH_4COOH gradient analogous to the elution pattern observed by Klenow[7] for Erlich ascities tumour cells. On pooling the fractions eluting as nucleosidetriphosphates (300 through 350 ml), 43.0 A_{260} units were obtained of the 420 A_{260} units applied to the column. Fractions containing the putative 3′-dATP were pooled, the NH_4COOH removed by chromatography on Dowex 50 (hydrogen from 2.4×11 cm) and the ultraviolet-adsorbing material eluted with water was concentrated by lyophilisation. Most of the product (94.4%) was resistant to oxidation with $NaIO_4$, followed spectrophotometrically by the method of Dixon and Lipkin[11], as expected of a deoxynucleotide. The ultraviolet adsorption spectrum of the product was characteristic of adenine ($A_{280}/A_{260} = 0.18$) at both acid and alkaline *p*H. 2.02 mol of acid-labile phosphorus was present per mole of adenine as determined by the method of Fisk and Subbarow[12] indicating that the product was a nucleosidetriphosphate. An anthrone derivative of the product was a nucleosidetriphosphate. An anthrone derivative of the product exhibited an absorption spectrum characteristic of 3′-dATP (ref. 13) similar to that of cordycepin and distinct from that of the anthrone derivatives of ATP or 2′-dATP. Finally, the sensitivity (91% inhibition at 22 mM) of DNA-dependent RNA polymerase from *E. coli* to the isolated product was equivalent to that exhibited by 3′-dATP on RNA polymerase from *Micrococcus lysodeikticus*[8]. The enzyme was insensitive to the naturally occurring 2′-dATP.

To demonstrate that specificity of inhibition by the phosphorylated derivative of cordycepin requires a template, we utilised the ATP–polynucleotidylexotransferase from maize seedlings. This enzyme catalyses poly(A) synthesis on single-stranded DNA as well as on RNA primers[15-16]. The specificity of inhibition by 3′-dATP observed when heated calf thymus DNA was used as a template in the RNA polymerase reaction (Fig. 1) was lost when the DNA was used as a primer in the exotransferase reaction (Table 3). The inhibitor specificity was not restored with poly(dT), as expected if the deoxyoligomers were used as primers and not as templates for poly(A) synthesis[15]. Analogous to the HeLa enzyme, poly(A) synthesis on tRNA by the maize enzyme was inhibited about 50% by either 3′-dATP or 2′-dATP at 0.22 mM.

Our *in vitro* results show that cordycepin *per se* has no effect on poly(A) or RNA synthesis unless in great excess of substrate concentrations. As in ascites tumour cells the drug is phosphorylated to 3′-dATP by HeLa cells. The lack of specificity for inhibition of two eukaryotic exotransferases by 3′-dATP renders the postulated *in vivo* inhibition of poly(A) addition to HnRNA unlikely (assuming ATP–polynucleotidylexotransferase catalyses *in vivo* poly(A) addition to HnRNA). The differential effect of cordycepin on HnRNA and mRNA synthesis *in vivo* may be better interpreted in terms of the preferential inhibition of HnRNA synthesis on dT-rich templates by 3′-dATP and subsequent limitation of RNA primer for poly(A) addition. The transcription of dT-rich templates would be consistent with HnRNA synthesis with low levels of actinomycin D *in vivo*. The recent finding of oligo(A) sequences in some HeLa HnRNAs (ref. 6) indicates that poly(dT) sequences are transcribed by eukaryotic RNA polymerase *in vivo*.

This investigation was supported in part by the US Atomic Energy Commission the National Science Foundation and the National Institutes of Health. G. M. was supported by a Medical Student Research Award from the College of Medicine, University of Florida.

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Different ultraviolet DNA endonuclease activity in human cells

THE first step of the model of the excision repair mechanism for ultraviolet-irradiated DNA is the incision of the DNA adjacent to the pyrimidine dimer by an endonuclease (ultraviolet endonuclease)[1]. Such endonucleases have been purified from *M. luteus*[2] and T4-infected *E. coli*[3] and partially purified from uninfected *E. coli*[4]. Human cells also perform excision-repair of ultraviolet-irradiated DNA[5] and ultraviolet endonuclease activity has been identified in extracts of HeLa cells[6,7] and human fibroblasts[7]. An ultraviolet endonuclease has been purified from rat liver[8]. But it has not been proved that any of these enzymes are true repair endonucleases, for the proof of an enzyme's role in cellular metabolism rests on analysis of its activity in mutants[4]. Cells from patients with xeroderma pigmentosum (XP) seem to be excision-repair defective mutants[1] and have been postulated to lack the ultraviolet endonuclease activity necessary to initiate repair[9].

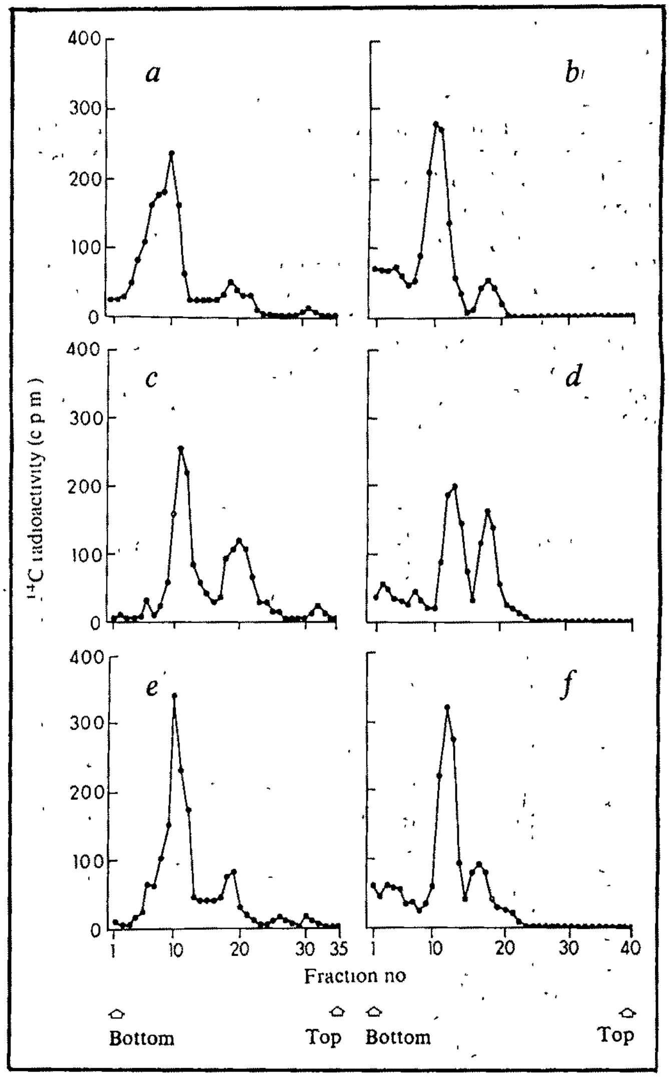

Fig. 1 Analysis of PM II superhelical DNA by sedimentation velocity centrifugation. Gradients *a* and *b* are profiles of 2-min ultraviolet-irradiated PM II DNA analysed by alkaline (*a*) and neutral (*b*) sedimentation velocity gradients. For alkaline gradients PM II DNA (0.1 μg) in a volume of 120 μl was layered on top of 4.0 ml of a 5–20% sucrose gradient, 0.001 M EDTA, 0.02% SDS, *p*H 13.0, with a 0.3-ml saturated caesium chloride cushion. Centrifugation was for 2.5 h at 40,000 r.p.m. in an SW 50L rotor in a Beckman ultracentrifuge (L2-65B). Neutral gradients contained the same amount of DNA layered on 3.0 ml of a 1.50 density caesium chloride solution, buffered with 0.01 M HEPES-KOH, *p*H 7.0, 0.001 M EDTA, overlayed with light mineral oil and centrifuged in the same rotor at 45,000 r.p.m. for 2.75 h. *c* and *d* are alkaline (*c*) and neutral (*d*) gradients of 2-min ultraviolet-irradiated PM II DNA incubated with HeLa cell enzyme preparation of approximately 2×10^5 cells under conditions identical to those described by Brent[6] in a volume of 120 μl. The reaction mixture was incubated for 20 min at 37 °C, chilled in ice, and then layered on top of alkaline and neutral gradients. Gradients (*e*) and (*f*) are the profiles of ultraviolet-irradiated PM II DNA incubated with the cell preparation of 2×10^5 XP skin fibroblasts (CRL-1200) and then centrifuged under alkaline (*e*) and neutral (*f*) conditions. All gradients were collected by the method of Carrier and Setlow[16] and counted in toluene–POPOP. Direction of sedimentation is from right to left. Ninety per cent of the applied radioactivity was recovered and all of it was under the two peaks.

Four genetic complementation groups have been identified among XP cells with rates of repair synthesis after ultraviolet irradiation that vary from 1% to 55% of control[10]. Using skin fibroblasts of each group, we have assayed ultraviolet endonuclease activity and found that all XP fibroblasts have similar activity. Control cells were two lines of normal skin fibroblasts, from subjects aged 3 and 31, a foetal skin fibroblast line, WI-38 foetal lung cells and HeLa cells. Ultraviolet endonuclease activity was assayed by measuring the conversion of ultraviolet-irradiated superhelical PM II phage DNA to the nicked form[6].

PM II phage was grown in host *Pseudomonas* BAL-31 by the method of Espejo and Canelo[11]. PM II DNA labelled with either ^{14}C or ^{3}H-thymidine (New England Nuclear Corp.) was purified by the method of Espejo *et al.*[12], followed by phenol extraction and dialysis against 0.1M NaCl, 0.02 M HEPES-KOH, *p*H 7.5, 0.001 M EDTA. After dialysis, the DNA was dissolved to between 15 and 40 $\mu g\ ml^{-1}$ as determined by 260 nm absorption with specific absorption assumed to be 0.020 $cm^2\ \mu g^{-1}$. Specific activity of the DNA was 2×10^4 d.p.m. μg^{-1}. Ninety per cent of the DNA was in the superhelical form as determined by alkaline sedimentation velocity gradients. Aliquots of DNA were irradiated by a single GE 15-W germicidal bulb (G15T8) from a distance of 15 cm for 2 or 3 min. This irradiation produced 40 or 60 pyrimidine dimers per PM II molecule as determined by the method of Carrier and Setlow[13].

HeLa cells were grown in Joklik modified minimal essential medium supplemented with 10% calf serum. WI-38 cells were obtained through the courtesy of Dr David Sabatini (Department of Cell Biology, New York University Medical Center). Both lines were determined to be free of *Mycoplasma* by culture and uridine phosphorylase assay[14]. *Mycoplasma*-free primary skin fibroblasts and XP skin fibroblasts were obtained from the American Type Culture Collection, Bethesda. Skin fibroblasts and WI-38 were grown on plastic dishes in Dulbecco's modified Eagle's MEM supplemented with 10% foetal calf serum in an atmosphere of 10% CO_2. XP and normal skin fibroblasts grew to 2×10^6 cells per 90-mm dish. Foetal skin fibroblasts and WI-38 cells reached 3×10^6–3.5×10^6 cells per 90 mm dish. WI-38 cells were received and assayed in passage 20–25. Normal and XP skin fibroblasts were received frozen in early passage and assayed within 5–10 subsequent doublings. Cells were prepared for assay by the method of Brent[6] at a concentration of 5–10×10^6 cells per ml. Protein was estimated by the method of Lowry *et al.*[15].

Brent assayed ultraviolet endonuclease of HeLa cells by measuring conversion of irradiated supercoiled PM II DNA to the nicked form by velocity sedimentation through alkaline sucrose gradients since, in neutral sucrose, complexes of the PM II DNA with the HeLa cell preparation prevented resolution of the two forms[6]. Thus, the breaks induced in the DNA could have been hydrolysis of alkali-labile bonds and the enzymatic event assayed a conversion to alkali-labile bonds rather than breaks in the sugar-phosphate backbone[6]. We resolved this problem with neutral caesium chloride gradients. Since caesium chloride had been used in purification of cellular DNA[18] we felt that aggregation of cell protein with PM II DNA would be minimal and the two forms of DNA could be resolved. Caesium chloride velocity sedimentation gradients had been used with polyoma DNA[17].

The gradients in Fig. 1 show that results were identical with alkaline sucrose or neutral caesium chloride gradients. The cell preparation did not attack unirradiated PM II DNA and 2 or 3 min of ultraviolet irradiation did not decrease the percentage of superhelical DNA. The demonstration of nicking of ultraviolet-irradiated superhelical DNA on neutral gradients proves there is ultraviolet endonuclease activity in the cell preparations.

Figure 2 demonstrates comparative activities of the different cell lines. HeLa cell activity increased from 12.5 to 75 µg of protein per reaction mixture. WI-38 activity peaked at similar protein content and was 70% that of HeLa. None of the XP or normal skin fibroblasts had activity greater than 40% that of HeLa, even at protein content as high as 240 µg.

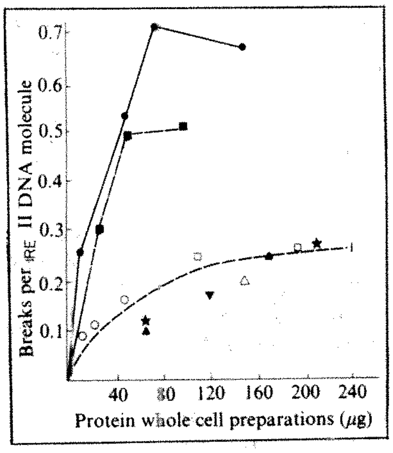

Fig. 2 Comparison of activities of cellular preparations. Conditions of enzyme assay were as described as in Fig. 1 except that the PM II DNA was ultraviolet-irradiated for 3 min. This increased amount of radiation did not in itself cause breaks in the DNA. But it increased the number of breaks introduced by all of the cell preparations. Breaks per PM II molecule were calculated from the Poisson distribution[17] which yields the formula number of breaks $= e^{-x}$ where $x =$ fraction of remaining superhelical molecules. The fractions of superhelical and nicked molecules were determined by summing the radioactivity under each peak. ●, HeLa cells; ■, WI-38; ○, foetal skin fibroblasts (CRL-1106); ▲ and ▼, adult normal skin fibroblasts (CRL-1121 and 1295); ★, △, □ and 1, XP skin fibroblasts from each of the four complementation groups. (CRL-1199, group 4) (CRL-1200, group 1) (CRL-1201, group 3) (CRL-1204, group 2). Each point represents the results of an individual gradient except for HeLa and WI-38 which are the average of two gradients.

These results demonstrate that human cells contain an endonuclease activity that breaks ultraviolet-irradiated circular DNA. This activity is present to a similar degree in XP skin fibroblasts from four complementation groups and three lines of normal skin fibroblasts. Thus, there is no correlation of activity with rates of repair synthesis. This would suggest that this activity is not the ultraviolet repair endonuclease.

The conditions of our assay, however, may not permit estimation of more subtle differences. In *E. coli*, ultraviolet endonuclease activity was the same in crude extracts of wild-type and excision-defective mutants[4]. This paradox was resolved by the demonstration of two UV endonucleases, one of which was absent in *uvrA* and *uvrB* mutants[4]. Therefore, a major endonuclease activity masked that of the true repair endonuclease[4]. Two ultraviolet endonuclease activities have also been identified in *M. luteus*[19].

Our results partially confirm those of Bachetti *et al.* who found no difference in ultraviolet endonuclease activity between one XP fibroblast line and a phenotypically normal XP heterozygote fibroblast[7]. They differ in that our HeLa cell activity was much higher than primary skin fibroblasts. This difference can be explained by the greater sensitivity of an assay using circular rather than linear DNA. This conclusion is supported by the similarity of our results to those of Brent[6].

This increased activity cannot be due to greater DNA content of HeLa cells nor their neoplastic origin since diploid, non-neoplastic WI-38 cells also have high activity. That this is a foetal characteristic shared by neoplastic cells is also unlikely since the primary foetal skin fibroblast activity was the same as adult and XP cells. It is unlikely that this elevated activity is an artefact of cell culture conditions since the foetal skin fibroblasts grew to similar density as did WI-38 and all primary lines were assayed during early passage.

We suggest that several ultraviolet endonucleases are present in human cells as in bacteria and that we measured a composite activity in whole cell preparations. Purification of these endonucleases may explain the relationship of this activity to cellular repair processes.

We thank Mr Archie Cummings for technical assistance. This work was supported by a grant from the US Public Health Service.

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Received January 30; revised March 9, 1975.

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Rolling circular DNA associated with Dane particles in hepatitis B virus

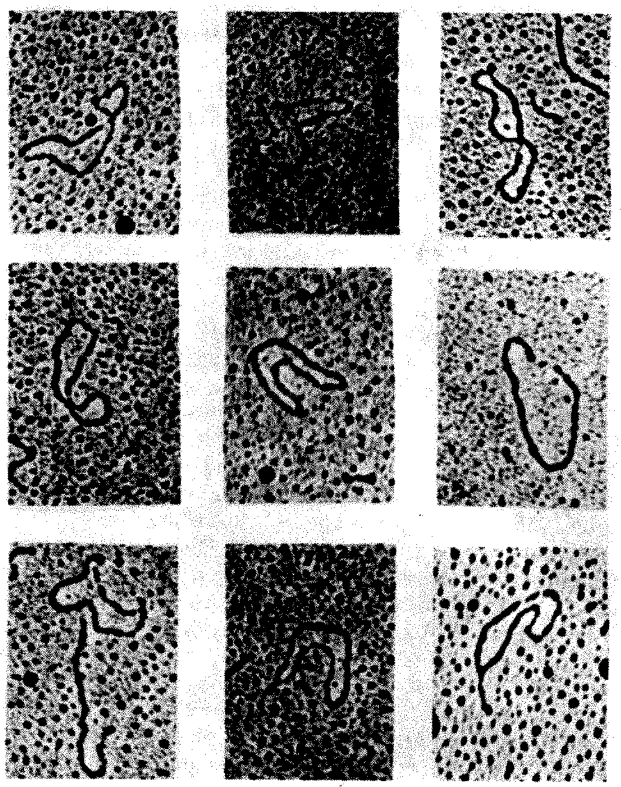


Fig. 1 Circular DNA extracted directly from purified Dane particles (×45,000).

PLASMA of hepatitis B antigen (HB$_s$Ag) carriers consists mostly of spherical particles of diameter 20 nm, together with a much smaller number of filamentous structures with the same diameter. In addition, larger spherical particles, 42 nm in diameter with a 28 nm core, first described by Dane *et al.*[1], are routinely found in crude plasma or purified preparations of HB$_s$Ag. Dane particles also contain a primed DNA polymerase activity[2] capable of synthesising new DNA. Robinson *et al.*[2] examined DNA from Dane particles by electron microscopy and found open, double-stranded circular configurations approximately 0.78 μm long. We have examined DNA isolated from Dane particles directly and also after formation of radioactive products by the endogenous DNA polymerase reaction. The DNA structures appeared in electron micrographs as double-stranded linear strands and closed circles, with examples of completely and partially open circles, and circles with attached linear segments in different lengths. The open circles with attached linear segments are suggestive of a replicative form, and this is consistent with a rolling circle model[4] for replication. This model for replication is strengthened by the comparison of the types of DNA found in particles before and after reaction of the polymerase. The number of rolling circles and the length of the tails were greatly increased after DNA synthesis. This type of replication has not been reported for animal viruses.

Plasmas from commercial blood donors, containing HB$_s$Ag of *ad* or *ay* subtype, were used to isolate and purify Dane particles by density gradient centrifugation and isopycnic banding techniques[2]. DNA polymerase activity and electron microscopy were used to monitor fractions during the isolation and purification. Three Dane particle preparations, shown to be essentially free of 20-nm spherical and filamentous particles be electron microscopy, were purified from approximately 6 l each of HB$_s$Ag-positive plasmas of subtypes *ay* and *ad*. One *ad* subtype and one *ay* subtype particle preparation, containing DNA polymerase activity, were then radiolabelled with ^{3}H-thymidine triphosphate (TTP) for 6 h at 37 °C. The reaction mixture, in a final volume of 12.8 ml, contained 1.6 ml of Dane particle suspension, plus the following reagents at the indicated concentrations: NP-40 detergent, 1%, mercaptoethanol,0. 3%; MgCl$_2$, 40 mM; NH$_4$Cl, 120 mM; Tris-HCl, *p*H 7.5, 160 mM; dATP, dCTP and dGTP, 0.5 mM each; and ^{3}H-TTP (17 Ci mmol^{-1}), 300 μCi.

Radioactive DNA was isolated according to the method of Kaplan *et al.*[2], and although there was insufficient nucleic acid to characterise by absorbancy, the total radiolabelled DNA recovered in the two preparations was 2.2×10^6 d.p.m. for the *ad* plasma and 3.6×10^6 d.p.m. for the *ay* plasma.

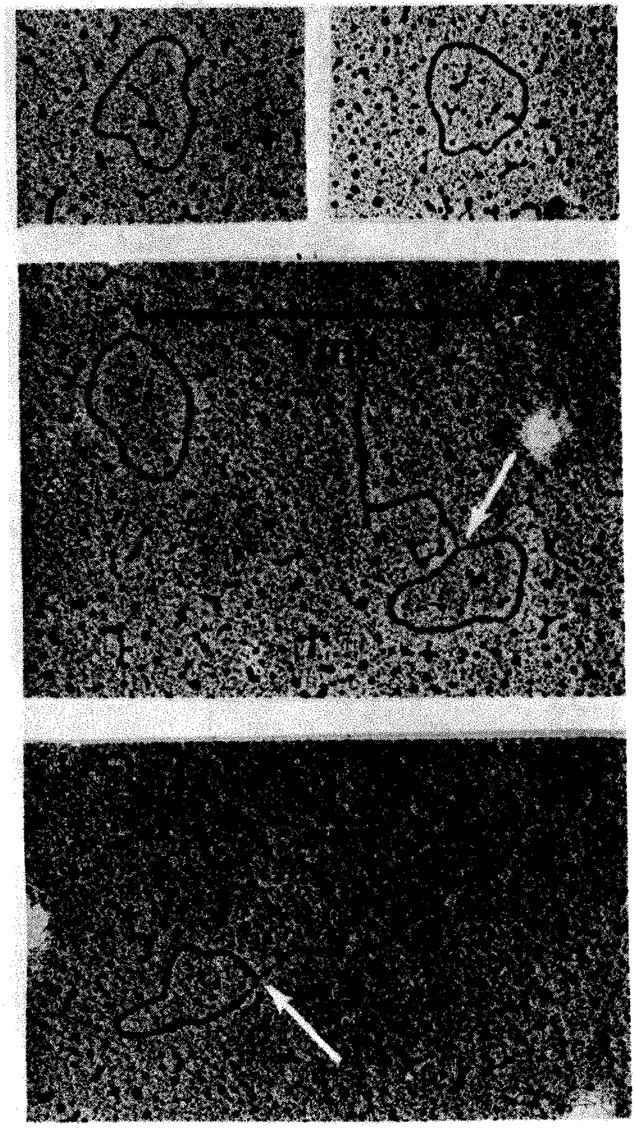
The third preparation (*ay* subtype) was divided into two portions. One was incubated for 16 h and extracted as above to yield radiolabelled DNA. The other portion was extracted directly to obtain native particle DNA. The four DNA preparations were submitted to one of us (T.K.) for electron microscopy using the protein monolayer with formamide technique[5].

Double-stranded open circles and linear strands were seen by electron microscopy in DNA extracted directly from purified Dane particles. The circular structures (Fig. 1) were approximately 0.78 μm in contour length, similar to the findings of Robinson *et al.*[3]. The linear structures (not shown) varied in length from 0.5 to 4 μm and there were many simple open circles. Three structures suggestive of tails were observed (Fig. 1, bottom row) in the Dane particle DNA. One of these (bottom right) appears to be an unequivocal rolling circle with a short tail.

The DNA configurations observed by electron microscopy were similar for the three preparations obtained after the polymerase reaction. The linear structures became shorter after incubation, suggesting possible degradation by endogenous nuclease. The most frequently observed structures were the double-stranded open circles and about 20 to 40% of these circular DNA molecules were found as readily identified rolling circles. These structures (Fig. 2) had linear portions with different lengths with an average of 0.55 μm, suggesting various stages of DNA synthesis. Several circles were found with tails longer than unit length. At the junction of the circle and the linear filament, a small single-stranded segment was observed (arrows, Fig. 2). The presence of tailed circles in rudimentary form in native particles, and an abundance of them after a DNA polymerase reaction, suggested that the polymerase-primed DNA in some of the Dane particles replicated by the rolling circle model[4]. This structure is based on one closed circle representing the negative strand to serve as a template for elongation of the positive strand, which grows by the addition of nucleotides to its 3′-hydroxyl end. The model for a double-stranded tail requires that complementary fragments are synthesised on the growing positive strand. The small segments of single strand at the junction of the circle and the linear filament would indicate the site of new synthesis of complementary fragments.

Although the infectious form of hepatitis B virus has not been identified, the Dane particle is a leading candidate for the infectious agent. The findings that a unique closed circular DNA and a DNA polymerase are associated with purified Dane particles are consistent with the hypothesis that a Dane particle containing nucleic acid is an infectious virion. The molecular weight of the circular DNA calculated from the mean contour length of 0.78 μm was about 1.6×10^6. This is similar to the findings of Robinson *et al.*[3] and indicates that the DNA is smaller than the double-stranded DNA of any known virus. The genetic information carried by DNA of this size is therefore very limited, and if this DNA represents the viral genome, some unique additional system, such as complementation or helper virus, may be required to programme the life cycle of the virus. Further studies will be necessary to establish whether the native configuration of the DNA is a superhelix or a 'nicked' open circle. Structures suggestive of superhelices (not shown) were observed, but could not be demonstrated reproducibly in these studies. The presence of an active, primed DNA polymerase in the native particles from plasma suggests the possibility of initiation of DNA synthesis in some Dane particles in blood or plasma. Although they were infrequent, short tails were observed in circular structures obtained directly from the particles before the *in vitro* incubation for DNA synthesis, indicating that initiation of replication by rolling circles had already taken place in a few particles. The quantity of rolling circles and the length of the tails increased significantly after incorporation of nucleotides. Thus, the *in vitro* polymerase reaction could result in elongation of pre-existing tailed circles as well as in new initiation of replication by rolling circles. The *in vitro* reaction is a highly artificial incubation condition, and therefore the rolling circles reported here may or may not represent the actual mechanism *in vivo*. Confirmation of the rolling circle mechanism of replication should be of interest because it would represent the first example known to us of this mechanism of replication of DNA obtained from a mammalian virus system.

Fig. 2 Circular DNA extracted from Dane particles after incorporation of ^{3}H-thymidine monophosphate with endogenous DNA polymerase (×45,000).



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Chemical and immunological differences between normal and tumoral colonic mucoprotein antigen

WE have found immunological and chemical differences between the colonic mucoprotein antigen (CMA) isolated from normal and tumour tissue. CMA is one of at least ten antigens reported from human colonic mucosa, one or more of which has been claimed to be tissue specific. Nairn[1] demonstrated histologically that one of these was a mucoprotein similar to the blood group substances, yet distinct and specific for the gastrointestinal system. Its clinical importance was emphasised by Bromberger and Perlmann[2], who demonstrated the presence of antibodies to it in the sera of patients with ulcerative colities. The differences reported here may be useful as diagnostic markers for colonic carcinoma.

The colonic mucoprotein antigen (CMA) accounts for

1% of the wet tissue weight and is chemically similar to the blood group substances but not antigenically related to them. CMA is also clearly distinct from the carcinoembryonic antigen (CEA) system. There is no major variation in the nature of the molecule between normal individuals although there is some microheterogeneity because of the size (1.5×10^7 daltons).

Tissues obtained either from surgery or at autopsy and stored at −70 °C until use were two primary adenocarcinomas (T1 and T2) and one metastasis from the liver (T3) from different patients. The tumour mucoprotein was purified as described previously for CMA[3]. Briefly, a phenol–water extraction was performed, the water layer was concentrated and fractional ethanol precipitation was carried out. The precipitate was subjected to gel chromatography on Sepharose 2B or 4B, the excluded peak being the product.

Elution profiles of the primary carcinomas on Sepharose 4B were identical to that of the normal tissue. There was an excluded peak at 28% bed volume containing the mucoprotein and an included peak at about 80% bed volume containing albumin, low molecular weight RNA and yellowish *E. coli* antigens[4]. The liver metastasis extract had a similar profile, without the *E. coli* antigens. The product yield of these tumours on a percentage of wet weight basis varied from one-third to one-half that of the normal tissue.

Antisera were prepared in rabbits, using purified material isolated from pooled normal colonic mucosa and the two primary tumours. A human red cell typing panel and antisera were obtained from Schering Diagnostics. Goat anti-CEA antisera (G100) was given by Dr J. Fierer of Francis Delafield Hospital, New York. Double diffusion studies were performed by the method of Ouchterlony[5].

Figure 1*a* shows the double diffusion reactions between antiserum prepared against normal colonic mucoprotein (centre well) and the normal and tumour-derived mucoproteins (peripheral wells). A single line is seen in each case. The tumour mucoproteins have reactions of partial identity both with respect to each other and with respect to the normal CMA. With this antisera it can be seen that the tumour mucoproteins do not all contain the same antigenic determinants and that the normal CMA contains determinants not present in either of the tumour products.

Anti-T1 antisera gave a reaction of identity with the three tumour mucoproteins (Fig. 1*b*), but no reaction with normal CMA. Anti-T2 antisera gave the same pattern (Fig. 1*c*), except for a spur on the T2 line indicating the existence of determinants present in T2 but not in T1. Again there was no reaction with normal CMA. This demonstrated an antigenic determinant (or conformation) specific for the tumour mucoprotein. None of the purified materials reacted with any of the blood typing antisera, nor did any of the prepared antisera agglutinate red cells from the typing panel. Goat anti-CEA antisera did not react with any of the purified materials.

Table 1 Amino acid composition and molar ratios

	CMA	T1	T2	T3
Thr*	17.4	4.0	5.9	2.6
Pro*	6.7	3.1	3.9	2.0
Val*	4.0	2.0	1.8	1.2
Ile*	2.3	1.1	1.2	0.7
Glu	5.0	4.9	5.0	3.3
Gly	3.9	4.2	3.9	3.1
Asp	3.7	3.6	3.4	3.7
Ser	3.7	3.5	3.8	3.1
Leu	3.3	3.2	3.0	2.5
Ala	3.1	3.0	3.1	2.3
Lys	2.3	2.4	2.9	1.8
Arg	1.8	1.2	1.3	1.1
His	1.4	0.9	0.9	0.7
Phe	1.3	1.3	1.4	1.1
Met	1.0	0.3	tr	—
Tyr	1.0	1.0	1.0	1.0

Samples were hydrolysed at 110 °C with 2.5 N HCl for 4 and 20 h and analysed with a Beckman Model 121 analyser. Cysteine and tryptophan were not determined.

*These represent significant differences between the examined mucoproteins.

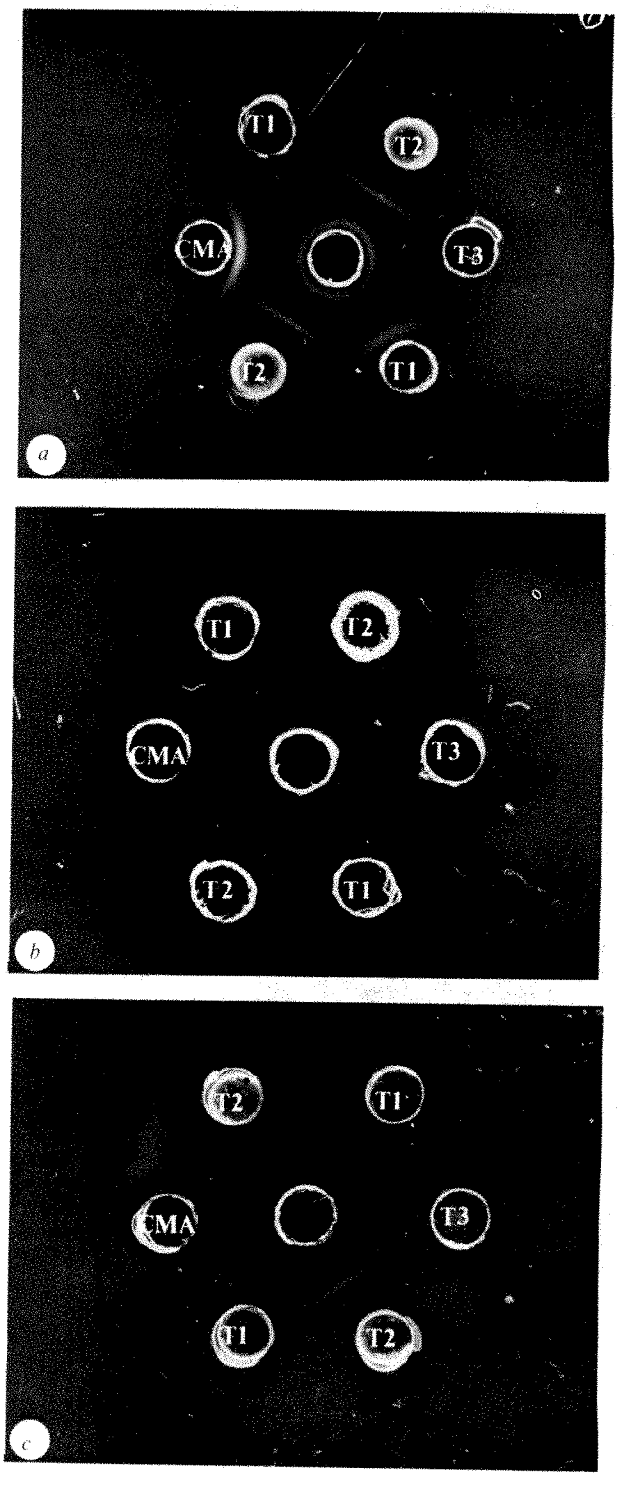

Fig. 1 Double diffusion analysis. Antisera in centre wells were: *a*, anti-CMA; *b*, anti-T1; *c*, anti-T2. Peripheral wells contained the purified mucoprotein from normal tissue (CMA) and the three tumours T1, T2 and T3.

Thus there is a clear distinction between CMA of normal and tumour origin. It must be stated initially that CMA is not made up of subunits. If this were the case, it might be argued that the tumour product could be a subunit analagous to the Bence-Jones proteins in multiple myeloma. Gel chromatography of normal CMA with phosphate-buffered saline, high salt or 6 M urea gave identical elution profiles. Reduction and alkylation in the presence and abscence of 6 M guanidine revealed no interchain cystine bridges The reduced and alkylated material eluted with the unreduced material when cochromatographed. More extensive analysis for subunit structure was performed on the equivalent material isolated from rat colon[6] No subunit structure was detected.

Amino acids were analysed using a Beckman Model 121 analyser after hydrolysis with 2.5 N HCl under a nitrogen atmosphere at 110 °C for 4 and 20 h. Extrapolated molar ratios (Table 1) were significantly similar for the four materials but there was less threonine, proline, valine and isoleucine in the tumour mucoproteins. The protein-carbohydrate linkage is O-glycosidic between galactosamine and threonine, possibly with a small percentage of prosthetic groups attached to serine in CMA. In many glycoproteins with this type of linkage, proline and isoleucine are found in close association with the site of linkage. Therefore it seems that the tumour cells synthesise at least some molecules similar in amino acid composition to those of the normal cell except that the linkage sites are absent.

Carbohydrate content was determined by gas chromatography of the alditol acetates[7]. Neuraminic acid analysis was performed after treatment with neuraminidase, by the thiobarbituric acid assay[8]. Paper chromatography[9] showed that the neuraminic acid exists as the N-glycolyl derivative The concentrations of the individual sugars varied from one tumour to the next, as Table 2 shows. T1 and T2 contained less carbohydrate than CMA, while T3 contained more. The molar ratios of T1 and CMA were similar while the other tumours showed greater divergence.

Table 2 Component analysis

	CMA (g per 100 g)	Molar ratio	T1 (g per 100 g)	Molar ratio	T2 (g per 100 g)	Molar ratio	T3 (g per 100 g)	Molar ratio
Fucose	7.03	7	3.92	5	0.83	1	15.99	15
Galactose	13.09	12	5 33	6	4.06	5	21.95	18
Glucosamine	11.36	10	8.63	10	7.88	10	11.91	10
Galactosamine	10.92	10	7.81	9	10.06	13	3.33	2
N-glycolyl-neuraminic acid	15.37	8	4.63	3	14.63	10	3.51	2
Protein	32.58		61.93		48.96		28.62	

Sugars were analysed by gas chromatography of the alditol acetates after hydrolysis in 1.0 N HCl for 1 h at 100 °C and 2.0 N HCl for 2 h at 100 °C. N-glycolyl neuraminic acid was determined by the thiobarbituric acid assay, protein using the amino acid analyser.

A larger group of tumours is being analysed to define this heterogeneity more exactly and perhaps relate it to antigenic differences. It may be analogous to that found in myeloma proteins, where there is a large degree of both central and peripheral heterogeneity in the carbohydrate prosthetic groups. Our results show that there is a distinction between the normal and tumour mucoproteins which may be useful in the detection of colonic carcinoma.

This work was supported in part by a grant from the National Institute of Allergy and Infectious Disease

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Received January 9; revised March 25, 1975

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Difference between the two iron-binding sites of transferrin

THE protein transferrin has a central role in iron metabolism, transporting the metal between absorption, utilisation, excretion, reclamation and storage areas. Although two ferric ions are bound at apparently equivalent iron-binding sites[1], the sites behave in a non-equivalent manner. Since the initial report by Fletcher and Huehns[2], there have been observations *in vivo* and *in vitro* of the unique biological specificity for transferrin iron bound at each site[3–8]. Physicochemical evidence for any difference between iron binding at each site is sparse. Price and Gibson[9] observed electron paramagnetic resonance (EPR) spectral differences arising from each site under the influence of the chaotropic agent perchlorate. Aisen *et al.*[10] observed EPR differences between sites for the chromium complex and Young and Perkins[11] reported that bicarbonate displaces only one oxalate anion from the (oxalate)$_2$–Fe$_2$–transferrin complex. We now report experiments on the *p*H-dependent dissociation of diferric transferrin which indicate that there is a difference in the iron-binding properties of each site.

Human transferrin (Beringwerke) was dissolved in water (250 mg per 25 ml) and dialysed[12]. The final dialysate was a solution of 0.010 M N-2-hydroxy-ethylpiperazine-N′-2 ethanesulphonate-0.001 M NaHCO$_3$, *p*H 7.45. Transferrin complexes were formed by mixing the apotransferrin solution with increasing amounts of 1.00×10^{-4} M tracer ^{59}Fe-labelled ferric dinitrilotriacetate, *p*H 6.0 (FeNTA$_2$). After incubation at 37 °C for 30 min, each solution was passed through a small buffer-equilibrated column (0.6×4 cm) of anion exchange resin (Biorad AG 1×4, Cl$^-$ form), and counted in a well-type gamma detector. Protein-bound iron was completely eluted from the resin, while iron in excess of the binding capacity was retained by the resin. The ^{59}Fe activity of eluates saturated with iron was used to determine the binding capacity and the transferrin concentration.

Four diferric transferrin solutions were prepared. Sample *A* was labelled with ^{59}Fe at *p*H 7.45. Samples *B* and *C* were mixed with ^{59}Fe (*B*) or unlabelled iron (*C*) at *p*H 5. *D* was not treated with iron at *p*H 5.00, *B* and *C* after removal of unbound iron were fully saturated with unlabelled and labelled iron respectively at *p*H 7.45 and *D* was saturated with ^{59}Fe at *p*H 7.45. Iron dissociated from these preparations as a function of *p*H was measured by resin absorption of dissociated iron.

Triplicate samples of apotransferrin were treated as follows. To sample *A*, at *p*H 7.45, was added 10% excess of ^{59}Fe–FeFeNTA$_2$. To sample *B*, adjusted to *p*H 5.00 with 0.01 N HCl, a 10% excess of ^{59}Fe–FeNTA$_2$ was added. To sample *C*, at *p*H 5.00, a 10% excess of unlabelled FeNTA$_2$ was added. Nothing was added to sample *D*, at *p*H 5.00. Each set of tubes was incubated and passed through resin columns which had been washed with HEPES medium at the corresponding *p*H. After determination of ^{59}Fe activity in samples *A* and *B*, 1 ml of 0.04 M HEPES–0.01 M NaHCO$_3$ solution (*p*H 7.45) was added to each eluate (3 ml), bringing the *p*H 5.00 eluates to *p*H 7.45. A calculated 10% excess of ^{59}Fe–FeNTA$_2$ was added to samples *C* and *D* to saturate the protein. Sample *B* was resaturated with a 10% excess of unlabelled FeNTA$_2$. The solutions were reincubated, passed through resin columns at *p*H 7.45, diluted to 10 ml (final transferrin concentration of 0.005 mM) and counted.

Sample *D* contained 97% of the activity of sample *A*, indicating that there was no loss of apotransferrin nor any alteration of binding capacity of the transferrin during these procedures. Sample *B*, initially labelled at *p*H 5.00 with ^{59}Fe and resaturated with unlabelled iron at *p*H 7.45, contained 41% of the activity of fully labelled transferrin (*A*). Sample *C*, labelled in the reverse manner, contained 62% of the activity present in *A*.

Samples of 1 ml from each of these solutions (*A*–*D*) were delivered into 0.5 ml of 0.10 M Tris-maleate buffers, *p*H 4.8–6.4. After standing for 45 min at room temperature, the contents of each tube were transferred to resin columns equilibrated with 0.01 M buffer at the corresponding *p*H. Tubes and columns were rinsed with three 0.5-ml samples of 0.01 M buffer, the eluates were counted, and the percentage of the total radioactivity was calculated. In these conditions, less than 0.5% of ^{59}Fe activity added to the buffers as $FeNTA_2$ or $FeCl_3$ was eluted from the resin.

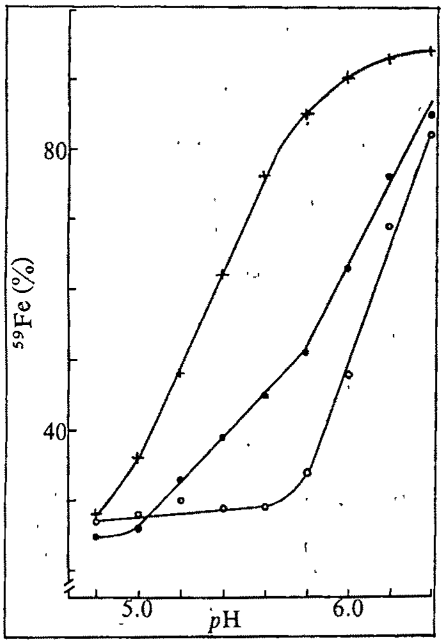

Fig. 1 Transferrin iron-binding in the *p*H range 4.8–6.4 Sample *A* or *D* (●) uniformly labelled ^{59}Fe diferric transferrin. Sample B (+) initially labelled with ^{59}Fe at *p*H 5, saturated with unlabelled Fe at *p*H 7.45. Sample *C* (○), initially labelled with Fe at *p*H 5, saturated with ^{59}Fe at *p*H 7.45.

Figure 1 shows that the percentage of ^{59}Fe bound to transferrin in the eluates from sample *A* or *D* (fully labelled with ^{59}Fe) as a function of the *p*H comprised two linear components intersecting near *p*H 5.8 where half of the metal was still bound to the protein. The ^{59}Fe-iron in sample *B*, which was initially labelled with ^{59}Fe at *p*H 5 (41% ^{59}Fe) and subsequently saturated with unlabelled iron at *p*H 7.45, was more stable to dissociation at corresponding *p*H than the fully labelled preparations (*A* and *D*). The dissociation was linear at *p*H 4.8–5.8. Sample *C*, which was resaturated with ^{59}Fe, also exhibited a simple linear relationship between the transferrin-bound ^{59}Fe but at *p*H range 5.8–6.4. This ^{59}Fe-labelled ferric ion occupied the site which did not bind iron at *p*H 5.00 and dissociated more readily at corresponding *p*H than the other samples when the *p*H was reduced below 5.7, 25–30% of the ^{59}Fe remained bound (*A*, *B* or *D*).

The biphasic nature of the *p*H-dependent dissociation observed with diferric ^{59}Fe-labelled transferrin suggests that there is a difference in the binding properties of each site at low *p*H which has not been reported before. Apparently one transferrin iron-binding site can bind a ferric ion at a lower *p*H than does the other site. Thus at *p*H 5, sample *B* bound the ^{59}Fe (approximately 0.8 mol of Fe per mol of transferrin) and sample *C* bound the unlabelled isotope. When the *p*H was adjusted to 7.45 and each sample was then resaturated with alternate isotope, 83% (1.0 of 1.2 additional iron) would occupy the second site. The dissociation data indicate that at *p*H 5.7 the uniformly labelled transferrin sample (*A* or *D*) still bound one ferric ion (50% of the ^{59}Fe). At this *p*H 32% (0.38 ^{59}Fe) of sample *C* and 85% (0.67 ^{59}Fe) of sample *B* was bound to the protein. The ^{59}Fe of *C* that was undissociated from *p*H 4.8–5.7 reflects that a portion of the ^{59}Fe occupies the more acid stable site. The metal binding characteristics are sigmoidal at high *p*H and when the *p*H is below 5.0.

If each site had equivalent iron-binding properties, when the complex was formed at *p*H 5, the ferric ions would distribute randomly between each site, and on resaturation at *p*H 7.45, the second isotope would also be distributed equally between both sites. Subsequent dissociation at low *p*H should not favour either site or isotope. The dissociation of ^{59}Fe in sample *B* or sample *C* should then be identical. This is not supported by Fig. 1.

The difference in iron-binding at *p*H 5 may arise with a conformational change to the protein such that one site becomes altered or even masked from iron-binding at low *p*H. When the protein reverted to its normal conformation at neutral *p*H, additional ferric ion would be bound at this unique, empty site. Although we have no information of such a conformational change, this mechanism could explain our finding.

There was a difference in the amount of iron bound by apotransferrin at *p*H (41% of the level at *p*H 7.45) and the amount dissociated from diferric transferrin in Tris-maleate buffer at *p*H 5 (26% of the level at *p*H 7.45) so that the data may not represent true equilibrium binding. However, the dissociation data agree with previous findings[13]. In studying the effect of *p*H on absorbance at 465 nm with ferrous ammonium sulphate and transferrin maintained overnight at 5 °C in the absence of buffers, Sturgenor *et al.* found that binding was complete when the *p*H was above 6.5 and was 50% of this value near *p*H 5.6, which is close to the value we observed (5.7) for 50% dissociation. The discrepancy between the association and dissociation binding values may be the result of differences in the ionic strength in each study, or may involve the participation of maleate in some manner. This, however, would have little bearing on our comparative dissociation study since all samples were treated similarly.

That one ferric ion, which is presumably bound at one specific iron-binding site, is dissociated more readily than is the other ferric ion could account for the biological utilisation of iron selectively from one binding site.

This investigation was supported in part by grants from the National Institute of Arthritis, Metabolism and Digestive Diseases and the Washington Heart Association.

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Received January 22, revised March 12, 1975.

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reviews

Imagery used to change behaviour

Ronald C. Lyle

Imagery and Daydream Methods in Psychotherapy and Behaviour Modification. By Jerome L. Singer. Pp. xi+273. (Academic: London and New York, 1974.) $16.50; £7.90.

THIS book attempts to straddle the chasm alleged to separate psychotherapeutic and behavioural approaches to the treatment of the 'mentally ill'. In a brave attempt to integrate widely differing approaches, Singer has sacrificed depth for breadth of coverage, and the overall impression is of a somewhat variable series of chapters rather than of an integrated whole.

The first chapter is, to a degree, bewildering; but by the second, I had begun to appreciate the literary style, despite some superficiality of content. Mention of philosophy is curiously confined to late 19th century North American philosophers/psychologists, and there is no consideration of the mind/body problem, nor even an account of the cognitive/behaviourist controversy within psychology. An interesting summary of the imagery methods currently used in psychotherapy is, however, included although it gives the impression of being a rather cursory overview.

The chapters on behaviour modification similarly lack a sufficiently detailed exposition of the underlying theories which Singer is seeking to replace with his own. Singer hits at the counterconditioning theory of desensitisation, but ignores the fact that most behaviourists have already moved to an habituation model. He mentions cursorily the use of deliberate exposure to real life situations, whether using low-intensity stimuli in the case of desensitisation, or prolonged exposure to highly anxiety-provoking situations in the case of flooding. Both of these procedures are now favoured in view of the difficulties which have been encountered with attempts to transfer to real life situations treatment carried out using imagined scenes. Although there are several techniques for using punishment to reshape behaviour, commonly based on electric shock, Singer devotes disproportionate space to the work of Cautela, who uses an imagined scene as the aversive stimulus. He also misses an opportunity to cite McGuire's work on the acquisition of deviant sexual behaviour by masturbation to fantasy imagery.

Singer follows this with a brief outline of experimental work on imagery, which relies heavily on self-report. Perhaps some mention of personal construct theory would have been relevant in this context. He then briefly outlines a theory relating affect and assimilability of information which seems to bear similarities to Pribram's better articulated concept of emotion as the degree of match/mismatch between stimulus input and expectancies.

A general theory of imagery is advanced which would allow the incorporation of psychodynamic concepts, in as much as early childhood experiences may provide generic categories for later information storage. In the final chapter, Singer rather hastily and unconvincingly discards conventional theories of specific procedures of behaviour modification for vague explanations in terms of cognitive restructuring.

I would not recommend this book as a comprehensive view of the imagery methods used in behaviour modification, although the summary of imagery methods used in psychotherapy is interesting. The book would have been more successful had it been more restricted in scope. ☐

Working in the cold

G. R. Pickett

Experimental Principles and Methods Below 1 K. By O. V. Lounasmaa. Pp. xii+316. (Academic: London and New York, September 1974.) £7.80; $20.25.

THE recent discovery of the existence of two superfluid phases of liquid ^{3}He below 2.6 mK has refocused attention on very low temperature physics. The field is at something of a critical point at present. The lowest attainable temperatures, which have been hovering around the 1–2 mK mark for more than a decade, seem about to advance into the microKelvin region, with all its interest of nuclear ferromagnetism, as nuclear cooling now seems to be less difficult than previously thought. It is thus a timely point for the appearance of a textbook on current experimental techniques below 1 K.

Lounasmaa's stated intention has been to write a book treating the four cardinal problems of low temperature physics: how to cool to low temperatures, how to measure the temperature when there, how to establish thermal contact and how to maintain thermal isolation The book keeps very close to this intent. The reader is led gently into the field with a discussion of the ^{3}He refrigerator before being introduced to the serious subject matter of the book which—in spite of the title—is experimental principles and methods below 100 mK The author has a clear expository style and deals with his subject in a straightforward manner. The chapter on dilution refrigerators perhaps makes these devices seem simpler than they are and a fuller discussion of some of the problems would have been helpful. Very little actual experimental work is discussed beyond that which touches directly on the problems of cooling, thermometry and thermal contact or isolation; and this restriction has limited the book to a manageable size. The one exception, which the author could not resist putting in, is the chapter on the superconducting quantum interference device, or SQUID, which, although not specifically a very low temperature device, is certainly useful in this regime.

The book is also a valuable source of reference. The chapter on thermal contact and isolation has a number of useful compilations of thermal conductivities and Kapitza resistances of commonly used materials. There is a very comprehensive author and subject index and an appendix of suppliers of low temperature instruments, equipment and materials, although this is likely to become rapidly out of date. Rigorous SI units are used throughout; pity is, however, taken on weaker brethren by the inclusion of conversion tables of SI to other units. A valuable book for any low temperature group to have access to and at the price not too expensive for the new graduate student to aspire to his own copy.

Directed illumination

Laser Physics. By Murray Sargent III, Marian O. Scully and Willis E. Lamb, Jr. Pp. xxvii+432. (Addison-Wesley: Reading, Massachusetts, and London, November 1974.) $13.50 paper; $22.00 cloth.

To an experimental physicist, some theoretical papers are memorable because they unify masses of phenomena into a theoretical structure and at the same time point the way to future developments. Such a paper was Willis Lamb's *Theory of an Optical Maser* published in the *Physical Review* in 1964. On one side, a satisfying structure was set up for analysing frequencies and intensities in multi-mode lasers. On the other, mode locking and interaction phenomena were suggested, and it could well be argued that the paper provided an impetus for the heated debate between 'classicists' and 'modernists', which has raged in quantum optics over the last few years.

This new text by Lamb and his co-workers was, therefore, awaited with much anticipation, and has lived up to that anticipation. It has succeeded in doing something which many texts fail to do: taking students in a pedagogical framework from undergraduate quantum mechanics and optics to the present boundaries of the subject.

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A discussion of atom–field interactions in terms of perturbation theory sets the scene and is followed by chapters on oscillators and on the maser. Density matrix formulation is discussed and is followed by the semi-classical laser theory, which was the subject of Lamb's 1964 paper, expanded and reorganised. Specific examples of the applications of the theory, such as gas lasers, are then treated. A chapter on the quantum theory of radiation leads into the quantum theory of the laser field and its fluctuations. Finally, appendices are provided to tidy away topics such as the modes of passive cavities and plasma dispersion functions which would break the flow of the main text. Examples and problems are provided at the end of each chapter and these are usually illuminating and often highly suggestive to the experimentalist. Sensibly, given the mass of laser literature, few primary sources are quoted, but the reader is referred to secondary sources for all necessary information.

It is fair to say that as a text book of laser theory it is unlikely to be surpassed. As the authors suggest, it could easily form the basis of a whole third year undergraduate quantum mechanics course, although there might be objections from nuclear and solid state lobbies. There are few textbooks which the average physics researcher can read through purely for pleasure, but this excellently produced and clearly laid out text may well become one of them.

D. G. C. Jones

Laser Light Scattering. By Benjamin Chu. Pp. xii+317. (Academic: New York and London, September 1974.) $31.50; £15.10.

A POWERFUL method of investigating the structure of non-crystalline substances is based on the study of the angular distribution of the intensity of scattered radiation. The history of this study dates back to Tyndall's experiments of 1869 and we may, of course, refer to the classical investigation of Lord Rayleigh on the blue light of the sky. The radiation used in scattering studies may be visible light (which is scattered without being absorbed by gases, liquids and suspensions or solutions of solid particles), X-rays or neutrons. We owe to Einstein and to Debye, in particular, credit for the theoretical apparatus which made it possible to determine the absolute molecular weight, thermodynamic interactions, and the size and shape of a variety of natural and synthetic particles or macromolecules of biological interest.

The advent of the laser has stimulated greatly the study of the spectral features of scattered light and the information concerning molecular structure which can be derived from this analysis; Brillouin first predicted in 1914 that such features would arise in liquids thermally excited by sound waves. By shifting the analysis from optical into radio frequency fields, light beating spectroscopy has allowed us to attain resolutions orders of magnitude greater than those accessible to classical spectroscopic techniques. The digital photoelectron autocorrelation technique has transferred the analysis from the frequency domain into the time domain, to which it is related by a simple transform. Spectral widths ranging from 1 to 10^8 Hz are now easily accessible through a number of relatively simple techniques (Raman scattering, which has also benefited greatly by the introduction of laser sources involves higher energy shifts). Developments within the last decade have shown that particle motions as diverse as the diffusion of molecules in Brownian motion, the chemotactic response of motile bacteria, the internal motion of flexible macromolecules or structures, or the velocity of wind, can all be analysed in terms of the spectral broadening in the frequency domain or the photoelectron autocorrelation properties of the scattered light.

Laser Light Scattering is timely because it attempts to present, in one useful integration, the various aspects, both theoretical and experimental, of this exciting new technique. I am not aware of a comparable source of information encompassing both practical and theoretical aspects of the problem in this rapidly expanding field. Yet the results of this heroic attempt have been only partially successful; too much has maybe been attempted in a book of a relatively modest size. The chemist and the biologist may remain somewhat bewildered by the fragmentary derivations whereas the physicist may expect a more advanced level of presentation.

Chu is, however, aware of that difficulty and provides enough references for additional detailed information. He discusses the intensity and the spectrum of the scattered light, the coherence properties and photoelectric detection of the scattered electric field, optical mixing spectrometers, Fabry–Perot interferometers and theoretical and practical aspects of photocount autocorrelation, lasers, optical systems, photomultipliers and data analysis. Finally, he reviews applications of the study of macromolecules, reaction kinetics, concentration correlation spectroscopy (with fluorescent probes), anemometry and critical opalescence. The book may indeed serve as a useful introduction to the interdisciplinary area of laser light scattering.

Henryk Eisenberg

Drug generalisations

Drug Actions on Cholinergic Systems. (Pharmacology Monographs.) By R. W. Brimblecombe. Pp. 227. (Macmillan: London and Basingstoke, January 1975.) £9.95.

THE first third of this book is devoted to a discussion of the action of acetylcholine like substances and their antagonists on peripheral receptors. Although it would be fatuous to deny that there must be some relationship between the chemical structures of such drugs and their pharmacological activity, it remains a secret relationship and structure-activity data have not provided the insights, perhaps over-optimistically hoped for 20 or so years ago. Some important advances, it is true, have been made, but these have been mainly in relation to the variation in the actions of different members of homologous series: at present it seems altogether too much to hope that the structures of receptors can be deduced from those of known agonists and antagonists. Unfortunately, however, it is precisely the not very convincing attempts to do just this, that the author has chosen to stress in his introductory chapters. One particular difficulty, of which the reader is not made sufficiently aware, is that in so far as agonists are concerned, biological activity is only partly related to affinity. The situation is in principle simpler with antagonists, but in practice the diversity of effective structures is discouraging.

The greater part of the book is devoted to a survey of effects which have been attributed to the actions of drugs on acetylcholine receptors in the central nervous system. Here too, I wonder if the selected literature, much of it concerned with behavioural changes which are not too well established is really worth summarising in book form. Perhaps statements like (p. 202) "... considered that it was not inconsistent with the hypothesis that nicotine stimulates the brain-stem activating system to produce behavioural arousal" cause arousal in some minds, but they are not likely to convey much to readers with a more general interest in cholinergic systems.

It is also difficult to accept that there is evidence to justify, even as a simple working hypothesis, the idea that all acetylcholine receptors in the central nervous system may be categorised as muscarinic or nicotinic, or that drug actions on behaviour may be described simply as stimulant or depressant (or of course biphasic); or (p. 194) that "nicotinic action is primarily on the brain stem and hippocampus while the muscarinic action is directly on cortical neurones".

The author is certainly well aware of the confusions and the controversies and the uncertainties of the present state of affairs. This does not, however, deter him from making rather large generalisations of the kind that are useful in elementary text-books. This seems to be a question of policy, since an extract from his general conclusions (p. 215) reads "... it would be premature at the present state of knowledge to attempt to explain the role of central cholinergic systems in anything more than the most general terms". Evidently the author does not agree that in pharmacology the particular must precede the general.

B. L. Ginsborg

Polypeptides

The Chemistry of Polypeptides. Edited by P. G. Katsoyannis. Pp. xiii+417. (Plenum: New York and London, 1973.) $34.20.

THIS book is a collection of essays on different aspects of peptide chemistry, written by eminent scientists in honour of Leonidas Zervas. Appropriately, the opening chapter by P. G. Katsoyannis describes the life and scientific work of Leonidas Zervas, from his being awarded the degree of PhD in Chemistry at the University of Berlin, to his retirement from the Chair of Organic Chemistry at the University of Athens in 1968.

Zervas's most remembered contribution to peptide chemistry is the use of carbobenzoxy protecting groups in peptide synthesis and this epoch-making discovery is stressed in the opening paragraphs of several chapters.

J. Rudinger, in discussing the relative merits of the even older tosyl protecting group (originally described by Emil Fischer in 1915), emphasises that peptide chemists should perfect the use of each available protecting group rather than abandoning it when an apparently better one comes along. Hirschmann and Veber contribute an interesting chapter on the concept and advantages of minimal protection in peptide synthesis, including the thiol group of cysteine Bricas appropriately describes, in view of Zervas's pioneering work on the synthesis of a glycopeptide, the present state of knowledge on the structure and synthesis of peptidoglycans and an unusual and interesting inclusion in a book of this kind is written by H. Hanson, on the mechanisms of intracellular proteolysis.

This book provides an historical review of assorted aspects of peptide chemistry with an extensive bibliography, but is not designed as laboratory text.

P. J. Lowry

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announcements

Award

Sören Jensen has been awarded the **Grand Prix** of the Swedish Royal Academy of Sciences for the identification of a polychlorinated biphenyl potentially dangerous to the environment.

Appointments

The University of Adelaide has appointed **Professor K. A. Provins** of the Department of Psychology in the Australian National University to the second of two Deputy Vice-chancellor positions. **Professor E. S. Barnes**, of the University's Department of Pure Mathematics, took up the other post at the beginning of this year.

Miscellaneous

Cancer study grants. The International Union Against Cancer is supporting the Yamagiwa–Yoshida Memorial Grants, designed to enable investigators to gain experience in special techniques in both the biological and clinical aspects of cancer research. Available only for study outside grantee's country of residence, as intended to encourage international collaboration (PO Box 400, 1211 Geneva 2, Switzerland).

International meetings

May 26–28, **World energy conference,** Copenhagen (DIS Congress Service, 36 Skindergarde, DK-1159 Copenhagen K, Denmark).

May 26–30, **Hot atom chemistry,** Louvain-la-Neuve (Professor Diapers, Laboratoire de Chimie Inorganique et Nucleaire, Chemin de Cyclotron 2, 1348 Louvain-la-Neuve, Belgium).

May 29–31, **Oncology and experimental models in cancer immunotherapy,** Bucharest. (Secretariat, Congress of Oncology, Boulevard 1 May 11, Sec 8, POB 5916, Bucharest 12, Rumania).

June 1–7, **Rheumatology,** Helsinki (European Rheumatology Congress, Box 30 00101 Helsinki 10, Finland).

June 2–5, **Smoking and health,** New York (Mr H. Milt, Staff Co-ordinator, 3rd World Conference on Smoking and Health, American Cancer Society, 219 East 42nd Street, New York).

June 23–July 4, **Radiation protection,** London (Dr H. D. Evans, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7, UK).

June 24–July 3, **Chemistry of solid–liquid interfaces,** Cavtat-Dubrovnik, Yugoslavia (IV Summer Chemistry Conference, Dr Velimir Pravdic, Rudjer Boskovic Institute, POB 1016, YU-41001 Zagreb, Yugoslavia).

June 30–July 4, **Entomology,** London (The Director, Commonwealth Institute of Entomology, 56 Queen's Gate, London SWY 5JR, UK).

July, **Pathological physiology,** Prague (Dr F. Palecek, Scientific Secretary of the Congress, Ke Karlovu 2, 121 09 Praha 2, Czechoslovakia).

July 2, **Mathematics of biological rhythms,** Cambridge (Secretary and Registrar, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-sea, Essex, SS1 2JY, UK).

Person to Person

Exchange of hospitality. Two sons and one daughter of Professor M. Sandler, aged twelve, eleven and ten respectively, separately seek hospitality in French household for two weeks this summer in exchange for return visits. All have some knowledge of the language (Queen Charlotte's Hospital, Goldhawk Rd, London W6 0X6, UK).

Leaf colour changes. Biologist visiting Canada during September/October this year would be interested to know whether any of the complex pathways for the production of compounds causing the myriad colours of leaves during the Canadian fall, have been elucidated in detail (Mr R Wylie, Loudoun Cottage, Whitchurch Road, Spurstow, Tarporley, Cheshire, UK).

There will be no charge for this service Send items (not more than 60 words) to Robbie Vickers at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted No commercial transactions.

Reports and publications

Great Britain

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nature

May 8, 1975

For those in peril on the sea

H. Russell Bernard, an associate professor of anthropology at West Virginia University, has spent six weeks aboard American research vessels under the sponsorship of the Office of Naval Research (ONR) studying the relations between crew and scientists. At the end of it he declares that "when they are not working together, they are working at staying out of each other's way. Physical isolation of crew and scientists was achieved by an elaborate time and space-sharing scheme . . . [which] may be seen as a manifestation of a gulf in values and behaviour in general". I wish the ONR had asked me; for half the price of placing an anthropologist on a ship I would have been happy to tell them that in the average research ship there are large spaces, even whole decks, that are no-go areas for either scientists or seamen. There are no notices saying "keep out"; the atmosphere is just not conducive to visitation.

Now there is, without doubt, good cause for someone to worry about scientist–crew relationships on oceanographic expeditions, one of the most expensive ways of doing research. The memory of the throwing overboard of Antarctic specimens kept in a crew's beer freezer is too vivid for complacency, and the crew of a ship cannot (with a few distinguished, even famous exceptions) be regarded as laboratory assistants or technicians in the land-based sense of the word. But what is really striking on most research cruises is not how the scientists and crew come to some sort of truce, but how deeply the scientists can dislike each other.

The reasons are not difficult to find. It is a certain sort of person who chooses to spend month after month at sea, and yet when he gets there he finds himself in the closest proximity, sixteen hours a day, with a similar sort of person, whose soggy cornflakes at breakfast are likely, in high seas, to end up in his lap. Data collection goes on all night, so each will have regular opportunities to awake the other in an indelicate manner at a hideous hour. Many experiments can only be carried out to the exclusion of all others, leading to endless bickering over the allocation of time. The mixture of disciplines—meteorologists, chemists, geologists, biologists, fluid dynamicist—which (on land, at least) is supposed to be good for science leads to blank incomprehension and a studied inability to see the point of anyone else's work. Some sea-goers work frantically all day and all night and cannot tolerate those who only appear for an hour a day. And one in ten of all oceanographers suffers from seasickness, frequently brought on by ships whose stability seems questionable. In the midst of all this stands the chief scientist, with problems not unlike those of an ice-hockey referee, and with the added complication that captains are always keen to get the ship back to port a day early.

The manifestations of these tensions are often bizarre. There is much practical joking of a pointed sort—a meteorologist may find his cabin completely filled with one of his balloons, and the presence of empty explosives cases allow for some pretty realistic and scary scenarios. The fighting is usually symbolic (water-filled balloons being very popular) but does on occasions end in a punch-up. The presence of so many tape recorders allows the alert discreetly to record the violent arguments that break out now and then, for replay at a quieter time. And accusations about the theft of tools never cease. Every oceanographer takes to sea with him a couple of dozen screwdrivers covered in complex identification marks and yet returns empty-handed and convinced that all his colleagues' locked toolboxes are stuffed with his property. When a research vessel goes to the breaker's yard, out of every crevice must come a screwdriver and a pair of pliers.

Amazingly, this physically unpleasant and emotionally conflict-laden environment not only exerts such a powerful attraction that sailor-scientists come back for more but also seems particularly fertile for the generation of new ideas. The best thinking in oceanographic science is often done on board ship in spare time rather than in the office back home, even though the best reception it is likely to get among one's shipmates is polite boredom. Perhaps a good way to revive some flagging scientific enterprises would be to transfer them lock, stock and barrel to a modest-sized converted trawler, to send them out to the mid-Atlantic and to require them to operate twenty-four hours a day.

There is plenty of sociological research left for the ONR to support. ☐

The little nipper who cost the South a fortune

Colin Norman reports on a threat to call a halt to a long and largely unsuccessful attempt to eradicate the fire ant.

THIS is an account of a fruitless attempt to block an invasion, of political manoeuvrings and fiscal blackmail, of chemical warfare and environmental destruction, and of open dispute between two factions in the federal government. Underlying the whole sorry affair are a variety of scientific issues, but they have generally taken a back seat.

The invasion began in 1918 when a small black ant, known as *Solenopsis richteri*, established a colony near Mobile, Alabama, after hitching a ride on a shipment of fruit from South America. Since then the ant and its red cousin *Solenopsis invicta* has invaded nine states from Texas to North Carolina, resisted a fearful onslaught of insecticides, soaked up $115 million in public money and stirred up a massive controversy.

For the past 18 years, the Department of Agriculture, in concert with the infested states, has been attacking the ants with a variety of chemicals, with the result that the programme has the dubious distinction of being the most expensive single pest-control effort ever carried out in the United States. But last month, Earl Butz, the Secretary of Agriculture, announced that the federal government is finally cutting its losses and will cease bombarding the ants with pesticides on June 30.

The announcement is probably more of a threat than a promise, however, for Butz's statement seems designed to bring political pressure to bear on the Environmental Protection Agency (EPA) to lift restrictions on the pesticide used to attack the ant. "Continuing restrictions placed on the pesticide", Butz said, "have finally made the program completely unworkable", and for good measure he added that the ants "could be totally eradicated with only negligible effects on the environment" if only the EPA were not so diligent in enforcing its regulations.

Officials of the EPA were clearly caught by surprise, for in 1971 the Department of Agriculture decided that for various reasons, including cost, a programme to eradicate the ants was no longer feasible. Future efforts would therefore be aimed at preventing the pest from spreading, the Department said. Nevertheless, Butz's statement has already promoted an outcry in the deep South, and some powerful southern Democrats are beginning to ask questions on Capitol Hill. The battle, clearly, is far from over.

Why has this insect provoked such a costly and seemingly futile campaign? The chief reason is that it has a nasty habit of attacking anybody who comes near it, inflicting a painful sting for which the creature has earned the name of 'fire ant'. It also builds mounds, sometimes reaching a height of about 3 feet, which have hard crusts and present a hazard to farm implements.

About 10,000 people a year were treated in Alabama, Georgia and Mississippi for fire-ant stings in 1969–71. Secondary infections and allergic reactions are the most common problems, although there have only been one or two reports of death from severe allergic reactions—in that respect, the ant is much less of a problem than wasps or bees. A committee which studied the fire-ant problem in 1972 reported that it is "of virtually no importance as a primary pest of crops, livestock or cattle" and in fact it may in some instances be helpful in killing off crop pests. The committee said, however, that the fire ant can harm agriculture by stinging farmworkers, to the extent that they will sometimes refuse to work in heavily infested fields.

The fire ant is clearly a great nuisance, but whether it merits a full scale chemical assault is, however, another matter. In any case, in view of the failure of past efforts to control the ant's spread, Butz's decision to terminate the programme seems like a prudent financial decision at a time when the Ford Administration is trying to reduce public expenditures.

The invasion began slowly at first, encompassing only about 2,000 acres by 1932 In the next 15 years, however, the fire ant had spread to about 2 million acres and by 1959 its territory had widened to 26 million acres. The federal government and the states began their effort to combat the ant in 1957, but the insect promptly responded by expanding its territory to 126 million acres between 1959 and 1969, which makes the anti-invasion strategy about as effective as the federal government's containment activities on the other side of the world. And, like the generals in Vietnam, the field commanders in the fire-ant programme were constantly promising that the end would soon be in sight.

In the early stages, heptachlor and dieldrin were used to bombard the fire ant at a cost of some $2 million a year to the federal government; a similar amount was supplied by the treasuries of the southern states. But, in 1962, the campaign switched to a new weapon—a chlorinated hydrocarbon called Mirex which was reckoned to create fewer environmental hazards and to be more effective than the other pesticides.

Described in one report as having "many of the requirements for an ideal control chemical for the ant", Mirex is applied in very small amounts—about 1.7 g per acre—dissolved in soyabean oil and spread on corn-cob grits. The grits are dropped on to infested fields by aircraft, and are taken up by foraging ants which carry the pesticide back into the mound, where the poison eventually wipes out the entire colony.

Although there had been a few attempts within the federal government to bring a halt to the fire-ant programme during the mid-1960s, the first major challenge came in 1970 in the form of a court suit brought by the Environmental Defense Fund (EDF), a Washington-based environmental organisation. Following reports that Mirex residues had turned up in a wide variety of organisms, particularly crustaceans and other aquatic wildlife, the EDF brought a suit to halt the programme. The immediate result was that on March 17, 1971 the EPA notified the manufacturer of Mirex—the Allied Chemical Corporation—of its intent to cancel registration of the pesticide. That first formal step to remove the chemical from the market was designed chiefly to open up a long investigation of Mirex, which was still going on when the Department of Agriculture made its announcement last month.

The first stage in the investigation was the appointment of a scientific advisory committee, which recommended to the EPA in 1972 that use of Mirex be continued to control the ants. The committee said, however, that the programme should not be aimed at eradication because "the environmental impact of such treatment is too poorly understood and the expense too great to justify a program of such magnitude". The EPA, therefore, decided to prevent the use of Mirex in coastal areas, heavily wooded areas and near inland waters. It also limited use of the pesticide to one application a year. Finally, the EPA began public hearings on the environmental impact of the programme in 1973, and those have been going on intermittently for the past fifteen months.

Although the EPA's regulations merely reflected what the Department of Agriculture was already doing in 1973, it was those restrictions which Butz condemned last month for crippling the fire-ant programme.

Why has the effort dragged on for so long, in spite of little success? The answer lies partly in the complicated politics of the deep South. For one thing, the $68 million that the federal government has so far pumped in to the programme (which has been augmented by $47 million from the states themselves) has provided a welcome addition to the coffers of state agriculture departments. And for another, the programme has powerful supporters in Washington, notably Jamie Whitten, chairman of the House appropriations subcommittee which handles the budget of the Department of Agriculture, and J. Phil Campbell, the Under Secretary of Agriculture. Whitten comes from Mississippi and Campbell comes from Georgia, both of which are heavily infested with fire ants.

Whitten's support for the programme in fact twice saved it from the axe in the mid-1960s, when the Bureau of the Budget tried to eliminate it. Whitten was furious and eventually increased the budget for the programme instead of eliminating it. By 1974, federal funding had passed the $7-million-a-year mark, and the Administration had asked for $9 million for the next year.

About 10,000 people a year were treated in Alabama, Georgia and Mississippi for fire-ant stings in 1969-71. Secondary infections and allergic reactions are the most common problems although there have only been one or two reports of death from severe allergic reactions.

The EPA's surprise at Butz's decision to pull out of the fire-ant programme is evident from a letter sent to Butz by John Quarles, Deputy EPA Administrator, a few days after the announcement was made. Noting that the Department of Agriculture had "abandoned the concept of eradicating the fire ant prior to the imposition of EPA restrictions on the advice of scientists that this effort would be financially and logistically infeasible", Quarles said that the announcement "tends to bring undeserved criticism to [the EPA] when other considerations appear to have played a larger role" in the decision to terminate the programme.

Replying to the letter, Under Secretary Campbell said that "we have chosen to exercise our prerogatives responsibly by suspending the program until we can operate free of crippling restrictions. We ask that, as you exercise your responsibility, you take into consideration that to be environmentally sound, the program must eliminate the fire ant as a pest".

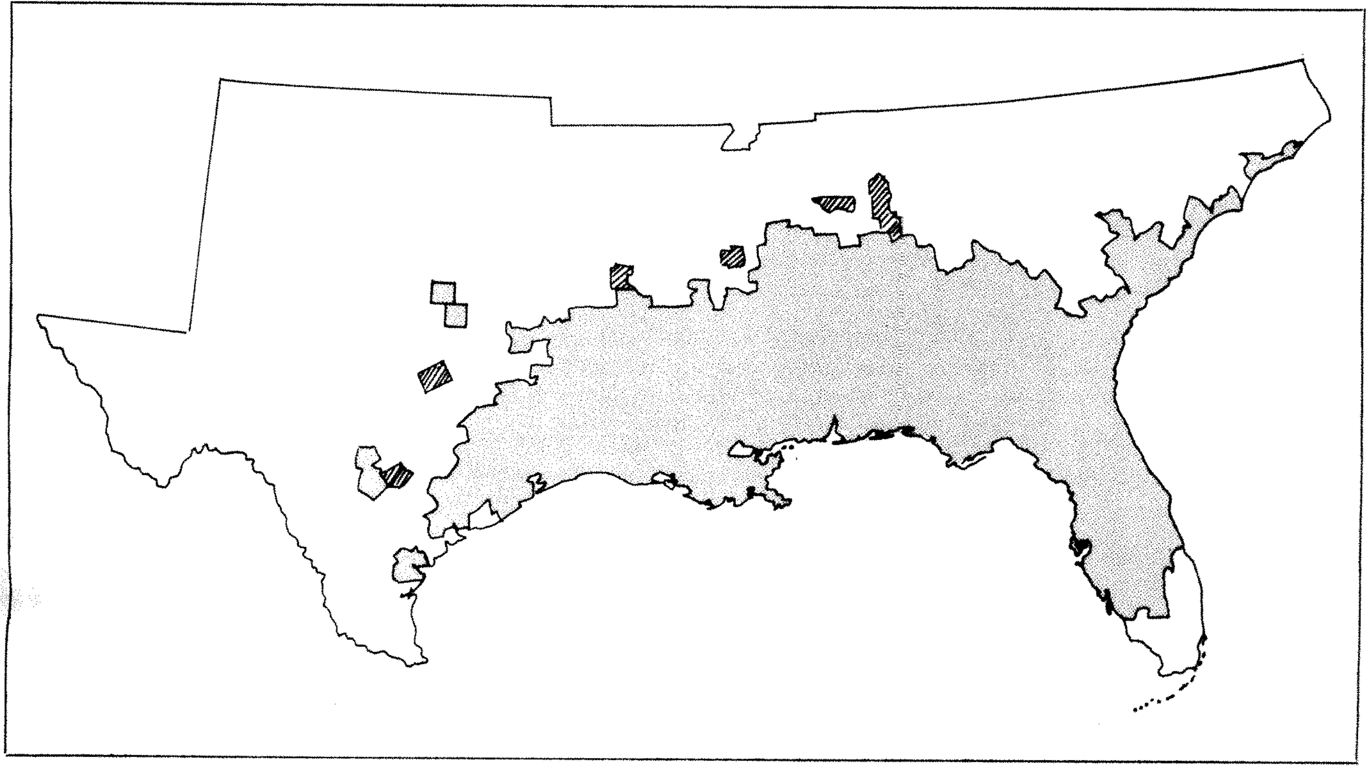
Asked last week what would be required to eliminate the fire ant entirely, scientists at the Department of Agriculture said it would probably take three applications of Mirex in an eighteen-month period over the entire infested area, including coastal regions. Since the federal government last year spent about $1 per acre on the programme, complete eradication would run into hundreds of millions of dollars.

The department's belief that eradication is possible is based on three large-scale trials carried out in Georgia, Florida and Mississippi in 1967, where almost total eradication of the ant was achieved after three applications of Mirex. Opponents of the programme point out, however, that the areas rapidly became reinfested, and that if such a programme is carried out on a larger area, the potential destruction of non-target species would be very large.

Nevertheless, Butz's decision has been greeted with dismay in the infested states, which have been relying on federal support for their efforts to control the ant. Typical of such reaction is a letter sent by the commissioner of Alabama State Department of Agriculture to Representative William L. Dickinson. "To deprive the inhabitants of the infested states of the program which gives some relief from the Imported Fire Ant is reprehensive [*sic*]", the letter stated.

It is unlikely that the plea will go unheeded in Congress, but exactly what will transpire is, at this stage, highly uncertain. □

Shaded area: generally infested area (eradication treatments not in progress or planned). Hatched areas: ant eradicated, and regulations removed.



Poisons, and how to dump them

The growth of concern about the tipping of noxious and toxic wastes in the UK has taken place against a background of increasingly tough legislation, the programme for which has nonetheless now come to a temporary halt as the result of a tight economic situation. John Newell reports.

DURING the next few months, the first reports are expected to emerge from technical groups within the Department of the Environment's waste disposal section. These groups are looking into the best ways of disposing of specific toxic wastes, including arsenic compounds, acids, mercury and other heavy metals in various concentrations, tarry residues and metal-finishing wastes.

Recommendations will take into account the 1974 Control of Pollution Act, which allows stringent requirements to be laid down for the disposal of particularly hazardous wastes. As soon as the Act comes into effect, all private operators will have to apply for licences for sites used to dump all noxious wastes. The power to withhold or remove licences, and the power to prescribe special codes of practice for especially toxic wastes provides local authorities with two powerful new safeguards. Nor do they any longer have the burden of proof of damage to the environment, as was the case under the 1972 Disposal of Toxic Wastes Act.

The 1974 Control of Pollution Act, the implementation of parts of which has been delayed for at least a year, goes further than the 1972 act in making it a duty for each local authority to make a survey of all industrial and domestic wastes created in its area. Local authorities must make detailed plans for the disposal of all wastes, and are compelled to introduce a licensing system for both landfill disposal sites and waste disposal operators. Local authorities will also be required to set out and maintain equivalent conditions for their own refuse disposal operations.

Although industry has welcomed the new act in principle, and has also supported the development of chemical treatment plants and modern incinerators, the economic situation has meant that the actual usage of such plants is somewhat disappointing, especially where smaller companies are concerned. But the implementation of the 1974 act should help those companies by ensuring that adequate disposal facilities are provided by local authorities.

For the foreseeable future, all existing means of disposal of toxic waste are bound to continue in use. The more recently designed chemical treatment plants for toxic wastes have, however, been designed with a view to increasing substantially the small proportion of their input which is at present recycled. Re-Chem International (which build chemical treatment plant) estimates that about 1 million tons of hazardous waste are produced annually in the UK, and this is thought to be increasing by about 1% (10,000 tons) a year.

The present need to restrict local-authority expenditure is inevitably delaying the timetable for bringing the act into full operation. Nonetheless, the parts of the act requiring local authorities to survey all waste, products and those concerned with the introduction of increased penalties for existing pollution offences, are being introduced over the next few months, as they require little or no extra manpower.

On the other hand, the requirement to produce a formal waste disposal plan has been deferred for a year, after discussion with local authority associations, and so also has the requirement to introduce the licensing system. The parts of the act relating to water pollution will also be implemented in phases starting next year.

The fact that the act is now on the Statute Book is, however, already causing alterations in waste disposal practice in preparation for what is to come. Landfill will remain the principle means of disposal and the Department of the Environment has had a research programme on landfill with toxic waste running since 1973. One aim is to produce a code of practice for use by waste disposal authorities in the selection and control of disposal sites. The code will be concerned with water pollution and atmospheric pollution hazards, odour nuisance, and contamination of soils and vegetation. The team involved is developing a mathematical model of the behaviour of hazardous materials in the interiors of landfill tips.

Sheer shortage of tipping space is already encouraging the recycling and reclamation of ordinary household rubbish and this will probably soon be the case for actively toxic wastes, too. The Department of the Environment points out, justifiably, that the 1974 Control of Pollution Act has been the model for an EEC policy directive and that UK legislation in this field is ahead of most of the world. But the pressure in the future seems bound to be directed towards more incineration, more chemical treatment to render harmless, and more active recycling of toxic wastes. □

international news

Foetal research ban to end

by Colin Norman, Washington

A PRESIDENTIALLY appointed commission will recommend later this month that the federal government should lift its moratorium on funding for research on living foetuses In place of the moratorium—which was imposed by Congress last year—the commission will recommend a set of stringent controls to ensure that such research is carried out according to strict ethical guidelines.

The commission's recommendations, which were agreed at a public meeting late last month, will be sent to the Secretary of Health, Education and Welfare along with a report providing justification for them. The secretary must either use the recommendations to draft regulations governing federal support of foetal research, or state publicly why he cannot accept them

The commission—the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research was established by Congress last year and was given four months in which to draft regulations governing foetal research and two years to draw up recommendations governing other types of human experimentation. The recommendations are, however, far from being the final word on the matter, because foetal research has unfortunately become ensnared in the loud and bitter dispute over abortion which has been raging in the United States for the past couple of years. The anti-abortionists are unlikely to accept the commission's recommendation.

The commission decided, in short, that therapeutic research, designed to benefit either the foetus or the mother, should be actively encouraged by the federal government, but that all foetal studies should be subjected to review procedures at the local level. One review committee would ensure that the study is ethically acceptable and likely to produce worthwhile results, while another would monitor the experiment to ensure that it is conducted according to ethical guidelines The mother's consent, and if possible that of the father, would also be required before any research on living foetuses could be conducted.

The two most controversial areas of foetal research—those involving studies of foetuses which have been scheduled for abortion but which are still in the womb, and experiments on living, but non-viable foetuses after they have been aborted—would require prior review by a national ethical review board, the commission suggests. Examples of the first category are the administration of drugs to pregnant women about to undergo abortion, and subsequent examination of the foetal tissue to see whether the drug crossed the placenta. Four researchers are, in fact, awaiting trial in Boston on charges arising from such an experiment. And the second type of research includes, for example, studies of foetal circulation.

Although the commission's recommendations are likely to play a vital part in the development of policies governing federal support of foetal research, they have already been made largely redundant in some parts of the United States. Biomedical researchers conducting some types of foetal studies in those places face stiff prison terms, and so for them the question of whether or not the federal government would support their research is entirely academic. ☐

ALTHOUGH it is common practice for elders of the scientific community to lament the decline in federal support for science, and to warn that the fabric of scientific research will be irreparably damaged unless the government adopts less parsimonious policies, a recent speech along those lines by Philip Handler, the President of the National Academy of Sciences, is drawing considerable attention.

Delivering his annual report to the academy last month, Handler noted that "there are scattered clouds on the science horizon" which include lack of growth in the science budget, threats to the peer-review system by which grants for basic research are awarded, and a "bookburning" attitude in Congress

Proceeding from the observation that the Ford Administration's budget for next year is essentially a "no growth" budget for basic research, Handler drew attention to the fact that "there are continuing pressures to maximize the use of available federal resources for the support and conduct of that science which promises earliest applicability". In particular, he suggested that "support of basic research by the Defense Department becomes increasingly constrained", and "emphasis on specific applied programs using funds formerly available for free research is a matter of policy at the National Institutes of Health, particularly the National Cancer Institute".

Those complaints are standard fare, but Handler suggested that "other factors loom as more worrisome", and "one can see in the offing a powerful threat to the peer-review system for decision making with respect to selection of research projects for support". The threat comes from the recent spate of press releases, emanating chiefly from the office of Senator William Proxmire, which have poured ridicule on science projects with trivial or funny-sounding titles. Such tactics, which are designed chiefly to generate cheap publicity, have planted in the public's mind "the seed of distrust in the judgement-making apparatus", Handler suggested.

Such distrust caused the House of Representatives last month to pass a preposterous amendment to the authorisation bill for the National Science Foundation to give Congress veto power over every individual grant awarded by NSF. Although the Senate is unlikely to follow suit, Handler noted that "the point is that the House came dangerously close to passing an amendment which was tantamount to bookburning". He continued, "the intrusion of political considerations in the award of grants by an agency whose principal purpose is to support the continuing dispassionate search for truth and to support education in that knowledge which has been gained by rigorous investigation according to the highest canons of our disciplines, is unacceptable". The House's action, Handler said, "is a giant leap backward by a supposedly egalitarian House which adopted a procedure appropriate only to authoritarian regimes".

Handler urged the scientific community to come to the defence of research projects that are subjected to attacks from Senators and Congressmen in search of publicity because if such projects "are not defended by all of us, it will be our turn next".

Soviet rocket puts India in space club

by Narender K. Sehgal, Jullundur

FOR the second time in less than twelve months India has forced her way in to an exclusive club, some of whose members happen to believe that she has no business being there in view of her poverty and food problems. First, it was the nuclear club on May 18 last year and then, on April 19, 1975, India's first ever satellite was successfully launched on a Soviet Intercosmos rocket—making her the eleventh member of the space club.

Prominent members of the scientific community have expressed happiness at the accomplishment. "This is another proud day for all Indians", said Dr M. S. Swaminathan, President of the Indian Science Congress Association, in Calcutta. Nearly everyone, while expressing satisfaction, said they were looking forward to the day when an Indian satellite would be launched from within India itself.

Named Aryabhata, after the famous Indian astronomer-mathematician of the fifth century AD, the blue and violet, 26-faced, diamond-shaped satellite weighs 360 kg and is orbiting the Earth every 96.41 minutes at an altitude varying between 564 and 623 kilometres and an inclination to the equator of 50.4°. The operational life of Aryabhata is estimated to be about six months, because that is how long the stock on board of an inert gas (used in the spin-up stabilisation mechanism of the satellite) will last. But the satellite is expected to stay up in orbit for about 2½ years. Aryabhata is equipped to carry out three scientific experiments during its operational life. One experiment, designed by ISRO (Indian Space Research Organisation) scientists themselves, will make X-ray measurements; another designed by the Tata Institute of Fundamental Research, aims at detecting high energy neutrons and rays at times of intense solar activity; and the third experiment, set up by the Physical Research Laboratory at Ahmedabad, will study electrons in the ionosphere and ultraviolet radiation in the night sky.

Work on Aryabhata began following an agreement between ISRO and the USSR Academy of Sciences in May 1972. (The USSR help was sought only for the launch.) The Indian Scientific Satellite Project, established by ISRO in December 1972 at Peenya near Bangalore, was given the overall responsibility for fabrication of the satellite. But the final product was the result of a collective effort by nine major institutions, including national laboratories, private and public sector undertakings and a number of small scale industries. The flight model was designed and fabricated indigenously and tested extensively both at Peenya and in the Soviet Union. Only certain components—the solar cells, chemical storage batteries, sophisticated onboard tape recorders and spin-up gas bottles—had to be obtained from the Soviet Union. Some 'space-qualified' electronic components, too, had to be imported from abroad because they were not available in India. The laboratories at Peenya included the space simulation chambers for testing the various sub-systems of the satellite. The chambers were designed and built by ISRO engineers in collaboration with the Bhabha Atomic Research Centre at Bombay and the Electronics Corporation of India Ltd at Hyderabad. The design and fabrication of instruments for commanding the satellite from ground stations at Sriharikota in Andhra Pradesh and Bears Lake near Moscow were also carried out by Indian scientists and engineers. The entire Aryabhata project cost Rs 43 crores (= Rs 43 million), including the infrastructure and facilities that went into creating it, and was executed in 30 months.

With this feat, the inevitable cropped up again. Among the first questions that the Prime Minister Shrimati Indira Gandhi and the Chairman of the Space Commission, Professor Satish Dhawan, had to face were variants of the following: "Why has India chosen to spend money on a space programme? How does Aryabhata fit in with the country's development priorities? Will there be any returns from these investments? Will they justify the monies and efforts expended on them?"

Fortunately these questions did not seem as difficult to answer as the ones posed about the nuclear explosion last year. For one thing, the objectives of Aryabhata are certainly not as controversial. Second, India has already been making use of foreign satellites, among other things, for communications. Moreover, a major educational experiment SITE (Satellite Instructional Television Experiment), to be launched in July this year, will make use of the American ATS-6 satellite for a year to beam television programmes in regional languages to receivers in several thousand villages in selected states.

Scientific and technological self-reliance is the under-lying theme behind the space programme, as is also the case with India's nuclear effort. The Indian government happens to believe that the nation's economic and social well-being will be helped by keeping abreast of the latest advances and developments in various scientific and technological fields. India, with the third largest scientific and technological personnel force in the world next only to the USA and the USSR, intends to make fuller use of space technology in the fields of communications, television, meteorological studies, earth resources, agricultural crop surveys and so on. Specifically, Aryabhata is designed as India's first step towards establishing her own capability in the area of satellite fabrication. The three scientific experiments, though important and "most modern" in the words of Academician Boris Petrov (Chairman of the "Intercosmos", the Soviet organisation in charge of international cooperation), are only of secondary importance; the mission would be considered successful if all systems aboard Aryabhata function normally, irrespective of the experiments. Happily, first indications are that all instruments and equipment aboard the satellite are working normally and well.

As a next step, Aryabhata 2 may be launched soon It will carry television camera systems to survey mineral

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THE Swedish and Russian Academies of Science have concluded an agreement on exchange of researchers and research. The agreement is unique in that, unlike the others the Russians have with western countries, it allows for the host country to invite specific scientists by name for study visits rather than accepting the other country's nominations. Each country will have 60 man months a year of expertise from the other, and the main areas of exchange are expected to be biology, physics and chemistry.

The agreement formalises and broadens already existing cooperation between Russian and Swedish scientists. Professor Hans Vilhelmsson at the Chalmers Institute of Technology, for example, has already had fruitful exchanges with Russian researchers in plasma physics. Last year he invited four specific Russians to a workshop at Chalmers. All came, and the meeting produced reports to be published soon in *Physica Scripta*.

Upholding the individual invitation clause will of course depend on goodwill between the scientific communities in each country and also on the Soviet government. There is plenty of goodwill at the moment, and an obvious incentive for the Russians at least to maintain theirs. For who knows what visiting Russian scientist may impress his Swedish hosts so much that they award him a Nobel prize. —*Wendy Barnaby*

Ice on troubled waters

from Vera Rich, London

THE new Soviet icebreaker Arktika has received, throughout her construction, the continuing press coverage reserved for major Soviet prestige achievements —interviews with the designers, scientists and research teams and a number of extensive, but often peripheral, articles related to the history of nuclear-powered icebreakers. To enhance this aura of prestige, the commissioning date of the Arktika was apparently chosen with an eye to symbolism: the hammer-and-sickle was hoisted on the new flagship of the icebreaker fleet "on the eve of May Day and on the threshold of the thirtieth anniversary of the victory over Nazi Germany".

Yet the Arktika and her sister icebreakers, which can extend the navigation season of the Northern Sea Route by several weeks each year and which undoubtedly rank as a major Soviet achievement, may well mark the quiet demise of an even more spectacular achievement *in spe*—the diversion of the Siberian rivers. This concept of a super-irrigation scheme, by which the waters of the Ob' and Yenisei would be "turned backwards" (conveyed by a series of canals to irrigate the deserts of Soviet Central Asia and, by way of the Volga. to replenish the shrinking Caspian) has been a dream of the Soviet planners for many years. The waters of the Ob' and Yenisei would no longer spill wastefully into the Arctic Ocean, but would have their directions of flow reversed, to the benefit of Soviet agriculture and the Soviet economy.

But now, likewise in the name of the Soviet economy, the construction of an icebreaker fleet implies another use for these waters. The home port of the fleet, Murmansk, is normally ice-free throughout the year. Some 1,500 miles eastward, however, lies the new mineralogical centre of Noril'sk, producing rich ores of non-ferrous metals. The only practical outlet for the products of Noril'sk, and the only means of providing its 150,000 inhabitants with essential supplies, is the rail link to the port of Dudinka on the Yenisei, and thence by river and across the Kara and Barents seas.

This river and sea route is normally blocked by ice for seven or eight months of the year. During these months, large icebreakers, such as the Ermak, the Lenin and now the Arktika must force a way through the ice for the freighter convoys. But these large ships (the Arktika is 140 m long and 30 m wide) can only operate between Murmansk and Dikson at the mouth of the Yenisei gulf. To conduct the convoys on the river route to Dudinka, "medium power" icebreakers (presumably with conventional engines) must be used.

If the Yenisei diversion scheme is still at the forefront of Soviet development plans, it would seem logical to assume that these smaller icebreakers are an interim measure and that, in the fairly near future, they will be replaced by a rail link from Dudinka to Dikson (which could, itself, rightly be considered a prestige achievement). This, however, does not seem to be the case. According to a *Novosti* release of March 10, "new up-to-date icebreakers with a small draught and able to negotiate the Yenisei are under construction". Although it is difficult to project the expected lifetime of these small-draught vessels, taking into account considerations of cost-effectiveness and the time still needed for construction, it would seem that the planners of the Northern Sea Route envisage that the lower reaches of the Yenisei will remain "navigable" (at least to icebreakers) until the end of the century. Which poses the problem: what becomes of the diversion scheme? Has it been quietly relegated into the sphere of plans that are desirable but not, within the foreseeable future, feasible (although still, from time to time, receiving the occasional tribute of press coverage?) or is there some lack of communication between the two planning bodies concerned—a division of interests, each demanding the use of the waters of the Yenisei in the service of the Soviet economy but pulling, literally, in opposite directions?

deposits and agricultural crops. The satellite—actually a back-up model for the one now in orbit—is reported to be almost ready. Encouraged by the success of its maiden venture, ISRO intends to begin negotiations soon with the USSR for a fresh agreement to launch it.

Satellite fabrication is only half the story however, as far as self-reliance is concerned. Work has also been in progress on a four-stage satellite launch vehicle at the Thumba rocket station in Kerala. But because of a shortage of funds, the project is running several years behind schedule. Moreover, it will only be able to launch a small payload weighing no more than 40 kg to start with—a mere ninth of the weight of Aryabhata.

It is just as well that the first Indian satellite was not launched from Indian soil, for possession of a rocket powerful enough to launch a satellite hundreds of kilometres into space would have most certainly been seen by critics aboard as an effort to develop an intermediate range ballistic missile capability. But here in India, one can discern no indication of any such attempt, if one goes by the small sums of money earmarked for the space and nuclear programmes—and the fact that the military has been kept well away from these programmes, almost to the extent of deliberate exclusion.

Notwithstanding hostile criticism abroad, there are many in India who believe she should keep all her options open, nuclear weapons and all, even if she does not intend to use them. A technical commentator for a national daily newspaper recently deplored the almost total lack of coordination between the nuclear and space programmes, on the one hand, and the military establishment on the other. He concluded thus: "Viewed from nearby and even with the bias natural to any one with the country's interest at heart, we are far from a nuclear weapons capability. Both the atomic energy and space research budgets are pegged at low levels. They cannot sustain a military capability in nuclear weapons. However, research projects must not, by deliberate choice, exclude the full range of applications of research achievements. Since both atomic energy and space research programmes are doing so, at least in the military field, Indian planning could do with some redirection, even at the risk of inviting further criticism overseas." ☐

US university R and D

THE US National Science Foundation has published a set of figures chronicling the decline in federal support for research and development in universities in the United States. NSF's figures show that total spending on research and development in higher education increased from $2.900 million in 1973 to about $3,000 million in 1974 which, when inflation is taken into account, represents a drop of about 5% in purchasing power. Within that total, however, federal support declined by 8% in constant dollars. Part of the decrease is explained by a book-keeping change involved in the transfer of the Draper Laboratory from the Massachusetts Institute of Technology into the independent non-profit sector, but even taking that into account, NSF points out that the purchasing power of expenditures on academic R & D declined by 3% and federal support declined by 6%. ☐

Great Lakes report

from Angela Croome

CANADA and the USA share in the ratio of about 2 : 3 the most extensive and one of the richest bodies of freshwater in the world—the Great Lakes system which consists of five lakes with a total surface area of 94,250 square miles. Not only are the lakes themselves large and valuable but they are surrounded by a region of gentle topography, temperate climate, vast mineral resources and bountiful farmland. Through the St Lawrence Seaway, opened in 1959, the lakes serve as a direct water link with the rest of the world, bringing ocean shipping to the heart of North America. As a result the system forms the hub of the North American population, with nearly 35 million people living in or near the Great Lakes Basin (one in every three Canadians, and one in every eight US citizens); predictions for the end of the century suggest a doubling of these figures.

When they were first discovered the vastness of the Great Lakes made them seem indestructible compared with the works of man. No longer. Their increased use, and rising industrialisation and population around their shores, makes the lakes system seem fragile in the face of man's multifarious interventions. Indeed they have proved so. Lake Erie was declared eutrophic some years ago. Both Lakes Michigan and Ontario have ceased to be safe for swimming and both have suffered serious algal blooms and fish-kills. Yet there is still a need to stretch the lakes' capacity to meet a swelling demand from the human population.

With the specific object of improving management of the Great Lakes as a whole to meet this demand, Dr D. V. Anderson, of the University of Toronto, proposed an International Field Year for the Great Lakes (IFYGL) as North America's principal contribution to the International Hydrological Decade which supposedly ends in 1975. Lake Ontario was chosen for this intensive study, because it is the smallest of the five (7,340 square miles) yet typical of the others (excepting Erie), convenient to study, situated in a growing population area and already showing signs of deterioration. The concept was that most of what was learned from the intensive study of Lake Ontario would be applicable to the other Great Lakes and even to other large lakes throughout the world. The project was delayed because of difficulties encountered in the USA, first in finding a 'lead' agency and then in obtaining the money for research. (Canada, with its centralised national research council structure and funding, was readily able to provide support by simply adding to ongoing projects.) The budget of $35 million called for was eventually collected and the IFYGL was initiated in April 1972.

Curiously there had been no co-operative agreement between the USA and Canada on protective and restorative measures until that date. In the rider to the agreement then made, the point was made that although the technology for repair was adequate, solutions to many of the problems could not be prescribed because knowledge and understanding of the various interactions within the systems are "woefully inadequate". Before the Field Year there had been plenty of individual scientific projects going on but there was no overall view taken. The six 'core' programmes selected—lake meteorology, energy balance, terrestrial water balance, lake water movement, biology and water chemistry—were intended to provide the data for sound solutions and wise future management. The publication of the preliminary batch of reports provides some opportunity to judge how successful this has been. Water quantity and water quality lie at the root of all the problems of the Great Lakes. The first shows up in such problems as flooding, eroding shorelines, low water in ship canals, storm damage to coastal facilities and cutbacks in production at the numerous hydroelectric plants. There is, in addition, the ice nuisance which both precludes inter-lake navigation in winter and often severely restricts the flow of water to the hydroelectric power stations. It has been suggested that waste heat from the increasing number of thermal electric stations be used to melt the ice, but this is a step to be taken only after weighing up all the other factors. Problems with water quality include sewage pollution, safe water supplies and, particularly important for Lake Ontario, the collapse of commercial fisheries. The unforeseen migration of alien species such as 'alewives' and parasitic lampreys from the Atlantic up the navigation canal network has played havoc with favoured game fish though overfishing met this menace half-way.

As it happened spring 1972 to spring 1973 was atypical in one respect: the weather. It was the wettest 12 months for years. Moreover on the basis of 30 years of records the driest month of the year is usually June but in the Field Year it was the wettest (by a factor of nearly two). Not surprisingly, the inflow and outflow to the lake was also abnormal, with the July runoff four times higher than usual. Whereas pathways and balance could be studied, the normal hydrological cycle was hardly manifest. The arrival of Hurricane Agnes during some days in June however, provided a bonus in the form of a thorough check of the reliability of rainfall radars. It was found out that the radars underestimated the volume of rain by as much as 40% and the errors were particularly marked when the heaviest rain occurred. Inadequate warning of floods is attributed to the radar errors.

A special study of the lake–atmosphere boundary layer by several means was incorporated in the programme partly because of the increasing interest this interface is attracting and partly because Lake Ontario offered a convenient site on almost a 'sea' scale. Preliminary results indicate that a large fraction of the annual evaporation occurs in a very few intense, cold-air outbreaks in autumn and that on these occasions there is an extremely large gradient of evaporation across the lake. A further curiosity identified by this programme was the occurrence from time to time of a wave-driven wind on the lake. (Some wave model studies carried out in 1966 had suggested that such a phenomenon might exist.) Specific studies of airborne sulphur dioxide contamination blowing in near Niagara (calculated to amount to several tons a year) showed that a lake with a *p*H near 8 may act as a very effective SO_2 'sink' for the atmosphere.

The study of fish population, which is of major significance to the Canadian authorities as they are in the midst of attempting to restore the ravaged Lake Ontario fish stocks, was specifically aimed to be a first step in a continuing programme to guide lake management. Collections of fish were made over the whole lake in all seasons except winter. A total of 63 species was collected and produced few surprises. Samples did, however, include a few deep water sculpin (thought to have been extinct) but no examples of the equally popular indigenous cisco. Introductions, the American smelt and the alewife, dominated the main trench and were also common pelagically. The authors end their paper with a heartfelt comment that "ecology is poorly understood in the physical aquatic sciences". This work is now a continuing 'core' programme for the Canadians, who hope eventually to defeat the invading lamprey and return their lake to full health and fish production.

THE constantly growing world shortage of non-human primates for research laboratories, which has already held up some Israeli immunological and pharmacological studies, will apparently lead to the development of a new branch in the economy of several kibbutzim—the raising of monkeys on a commercial basis. Such a scheme has been in the air since the early 1970's when the National Council for Research and Development sponsored a feasibility study. But it was rejected three years ago because it was still cheaper at that time to import non-human primates from countries in Latin America, Asia and Africa (where they only had to be trapped) than to raise them in captivity, in itself a risky venture.

The cost of monkeys on the world market has, however, significantly increased in the interval and, more important, they are not always readily available even for buyers willing to pay the ruling prices At the same time, a number of kibbutzim have succeeded, without fanfare or official sponsorship, in raising such animals on a modest scale. Local collective settlements originally brought in monkeys for their special educational farms, where kibbutz children cultivate crops and raise animals on their own, with a minimum of adult supervision. Most of these primates adjusted well to kibbutz life and in some cases even multiplied.

Thus it was natural for Israeli primatologists to view the kibbutzniks as potential partners in a scheme to raise non-human primates on a larger scale for local laboratories and perhaps for export. Initial plans call for the creation of several pilot centres, in places ranging from the relatively cool Galilee to the sometimes very hot Negev. Their first inhabitants will be rhesus, vervet and squirrel monkeys, the progeny of which, if all goes well, will be available for marketing in 1978.

It will be a long time before the kibbutzim can produce enough monkeys to meet the requirements of local laboratories (about 500 animals a year at present), let alone have a surplus to sell overseas. But if the scheme proves successful, it will be of great benefit to research scientists here as well as a welcome source of income for the kibbutzim, particularly since kibbutz children will do most of the routine work that does not involve physical contact with the animals.

There is however, still some opposition to be overcome at the settlements themselves One kibbutz teacher, for example, is concerned with the possibility that raising the primates will create educational problems "It is well known that many monkeys in captivity spend a great deal of time masturbating in their enclosures. How", he asked me in great bewilderment, "am I going to explain that to my first graders?"

- Links exist between Israel's kibbutzim and her institutions of research and higher learning on many levels. Blessed with a highly intelligent population, the settlements are quick to use research results relevant to their industrial and agricultural branches, as well as to their educational system. Moreover, some 2,000 university students and almost 100 university faculty members are from kibbutzim.

Letter from Israel

from Nechemia Meyers, Rehovot

HEATH
DO NOT WATCH THE MONKEYS

Although few participants were aware of the fact, the man who organised this month's EMBO Workshop on muscle cell culture in the study of gene expression during differentiation, Professor David Yaffe, of the Weizman Institute is a member of Kibbutz Givat Brenner. This same collective settlement boasts yet another Professor at the institute, Achi Brandt, the mathematician. Head of the Pure Mathematics Department, Professor Brandt contributes not only his salary but also his know-how to the kibbutz, helping it to use computers in planning the most efficient exploitation of its manpower and production facilities.

As Brandt recently explained it: "To establish the best method of distributing kibbutz resources. you must first assess the possible economic activities, their income and cost, and the availability of such components as manpower, land, capital and water. At the same time, you cannot overlook tricky agro-technical, seasonal and even ideological restrictions (such as the desire of most kibbutzim to minimalise or, if possible, eliminate the 'exploitation' of hired labour in kibbutz fields and factories). With these data, the optimal plan can be found by a properly designed computer program which can also tell us the relative importance of each restriction imposed on the system."

Brandt even showed his fellow kibbutzniks how a computer could be used to help the settlement reach decisions on social issuses. "For instance", he told me, "we have long had a fierce dispute as to whether our children—who now all sleep in what we call children's houses—should not perhaps sleep at home with their parents. So I devised a questionnaire to sift out actual needs from mere opinions, and wrote a program which analysed the answers. We discovered that, quite contrary to what we thought, only a minority of kibbutz parents really feel that their children should sleep at home. At the same time, this minority wants to change very badly, so that some modification in the system may very well occur at Givat Brenner as it has at other settlements."

- The unique ethnic character of Israeli kibbutzim has long attracted the interest not only of the sociologists and anthropologists but also of medical researchers, who welcome the opportunity to measure the positive and negative effects of life in Israel on the health standards of various communities. Some years ago they discovered for example, a clear link between the onset of Westernisation—particularly the adoption of Western eating habits—and an increase in the incidence of heart disease and diabetes among Jews from Yemen, who, before their emigration, had lived in a 14th century society and subsisted on a virtually fatless and sugarless diet. As they began eating more fats and sugars, they grew increasingly prone to heart disease. Of course one shouldn't overlook the fact that their life expectancy here is still much higher than it had been in Yemen, where modern medical care was unobtainable.

Now the same pattern has developed among the Bedouin. Doctors at the Negev Centre Hospital in Beersheba, where hundreds of Bedouin are treated each year, report a steady increase in heart disease among them, even though it still remains much lower than among the Jewish residents of the area. But, as with the Yemenites, overall life expectancy has risen to West European standards (70+ for males), by comparison, for example, with 50 years in neighbouring Egypt.

Israel's best known ethnic malady is Tay-Sachs Disease, a fatal genetic disorder limited almost entirely to infants whose forebears came here from certain parts of East Europe. Although the disease is still incurable, Israel—like Jewish centres in the USA and Canada —has instituted a Tay-Sachs screening and counselling programme to advise potential parents whose offspring might be affected.

Jews from Oriental lands also have their 'own diseases', one of which—favism—was responsible for a completely unnecessary mini-panic here recently. The hereditary disease strikes people who lack a particular enzyme (G6PD), one which protects red blood cells against the noxious effects of the fava bean and other materials, and thus prevents anaemia. It is by no means limited to Jews, as it affects, among others, Scandinavians, American Negroes and groups in South-east Asia. But among Jews it is limited almost exclusively to people who have lived in Iraq, Kurdistan or Iran.

In spite of this well established fact, the Ministry of Health last week dramatically announced that all Israelis should refrain from eating fava beans (which, together with common medicines like sulpha drugs and aspirin, trigger favism). The sale of fava beans diminished, of course, but rose again when citizens were informed that unless they came from those three countries they could eat the lentils to their heart's content. A screening programme now being carried out at local hospitals should clarify the problem for all Israelis in the course of the next few years.

- The dangers of inbreeding within a particular ethnic group are starkly emphasised by the problems facing the Samaritans, an ancient Holy Land sect which now numbers some 450, half of whom live in Holon, a suburb of Tel Aviv, while the other half live in the West Bank town of Nablus. Becaus 58% of Samaritan marriages take plac between members of the same famil an abnormal number of physically o mentally handicapped children is bor to members of the community. Ne blood in the form of five Jewish bride has improved the genetic mix in recen years, but the community still remain dangerously inbred.

Yet these people, descendants of th Good Samaritan, show an extraordinary will to survive. They no number more than three times a many as they did a hundred years ag when *The Times* reported that ther were only 135 Samaritans in the entir country. □

correspondence

Mediterranean mercury

SIR,—The article by Alexander Dorozynski (April 17) on the "Mediterranean poison fish forecast" made by Dr Maurice Aubert, Director of the Centre d'Etudes et de Recherches des Biologie et Oceanographie Médicale in Nice states that more than 1 p.p.m. of methylmercury is present in nine species of fish caught in the Mediterranean. There is a calculation attributed to Dr Aubert worth quoting: "Since it has been found that only 4 to 5% of mercury absorbed is fixed in the organism, the weekly absorption of food containing 2 mg of mercury will result in a fixation of 80 μg. At this rate the lethal dose is reached about 20 years". The lethal dose according to Dr Aubert is 80 mg, which is reached in the fisherman by the following equation:

$$2\ \text{mg} \times 0.04 \times 52 \times 20$$

This calculation has no scientific basis. First Japanese data indicate that 80 mg is the lowest toxic body burden and not the lethal dose. Second abundant data reviewed by expert committees show that approximately 95% of the ingested methylmercury is absorbed from the gastrointestinal tract and that the average half life of methylmercury in the human body is 70 days. This means that the body burden of the fisherman in question will be 24 mg after 6 months, 28 mg after 1 year and 28.86 mg in the steady state when he will excrete exactly 2 mg of mercury a week.

The same criticism applies to Dr Aubert's calculation for the child victims of Minamata. He also misquotes the lowest level of hair mercury associated with intoxication. According to the work of the Swedish Expert Group on the Minamata epidemic, 60 μg/g Hg signals the danger of intoxication rather than twice the level (7.39 μg/g) found by Dr Aubert in a fisherman. Based on the relationship between the concentration of mercury in hair, the blood level, the body burden and the weekly dose, his fishermen probably had a body burden of less than 10 mg, which assuming steady state conditions was the result of the weekly consumption of 0.7 mg mercury as methylmercury.

Yours sincerely,
L. MAGOS

MRC Laboratories,
Carshalton, UK

In line 9 of paragraph 5 of the article, 25 μg should read 25 mg.

Plants

SIR,—Rain forests, vanishing wild life and the destruction of rare wild flowers by reservoirs are all sure of space in periodicals and funds for conservation on grounds of preserving the gene pool, yet garden varieties of vegetables are in greater danger of extermination. The FAO are now searching Turkey for hardy varieties still grown by peasants, but Europe is destroying her genetic heritage from the best of motives.

Modern methods of seed storage make it possible to keep packets "in mothballs" for as long as 200 years and I suggest that stocks of all deleted varieties should be donated to a National Seed Library, ideally sited at a University such as Reading, from which they would be available to plant breeders and enthusiasts. We would be glad to hear from those who are seriously interested in this project an from Foundations and others wh would make grants to start a Worl Vegetable Fund, like the World Wildlife Fund, but more urgent in view o the greater pace of extermination.

Yours sincerely,
LAWRENCE D. HILLS

Henry Doubleday Research
Association,
Braintree, Essex, UK

Methinks he doth edit too much

SIR,—Every new scientific journal t appear sports a long list of associat editors, members of the editorial board advisory editors or whatever. These ar generally academics who are presume to guarantee the quality of the product my suspicion is that they rarely do anything beyond collect an annual fee referee a paper or two and line thei shelves with complimentary copies o the journal which they don't read. On journal that I see has an advisory boar of six in addition to twenty or thirt associate editors; all the advisors hav a Nobel Prize but only one in a fiel close to that of the journal.

I don't begrudge a fellow one sinecure, but it is my impression that som hold five or ten editorial positions. Ho about a prize for the man with the mos appearances?

Yours faithfully,
M. CHARLES

Boston, Mass

A year's free subscription for he or sh whose nominee tops the list—bu nothing for the nominee, whose shelve are probably full already. Entries b July 1—Ed.

news and views

Chinese work on insulin

from Dorothy Crowfoot Hodgkin

Two very beautiful papers on insulin appeared in *Scientia Sinica* in December 1974 (**17**, 752 and 779). They continue a sequence of scientific publications which began in 1961 and includes the total synthesis of sheep insulin, described in 1965. They are published by groups of research workers in China: in Peking in the University (Departments of Biochemistry and Organic Chemistry) and in the Institute of Physics of the Academia Sinica, in Shanghai in the Institutes of Organic Chemistry, Biochemistry and, most recently, Zoology. They provide impressive evidence of integrated researches in different disciplines and laboratories.

The first of the present two papers describes the derivation of an electron density map of insulin in rhombohedral 2-Zinc insulin crystals at 1.80 Å resolution. It gives useful experimental details of the preparation of the crystals and heavy atom derivatives, of the measurement and interpretation of the X-ray diffraction data and of the stages of solution of the structure. The model and electron density map (clearly of very good quality) are illustrated in colour and the atomic arrangement is described in detail. Ramachandran plots and a plot of the distribution of $C\gamma$ side chain atoms viewed along $C\alpha$–$C\beta$ illustrate some of the stereochemistry observed.

The structure is essentially the same as that found in parallel crystallographic studies in Oxford; indeed, in 1972, in Peking, we compared in detail our earlier electron density maps, both drawn at a scale of 1 cm to 1 Å. There was actually a moment of acute concern when we put the maps together first and they did not fit—until we realised one was upside down compared with the other. We then saw that there was very good correspondence, if not exact agreement, between them. Correspondingly now, in the insulin dimer, molecule I Peking is molecule II Oxford, and molecule I Oxford is molecule II Peking.

One might not wish, in all cases, to see complete duplication of the X-ray analysis of a protein molecule—so much work is involved. But there are great gains in the present case from having two views of the insulin crystal structure. First, insulin is such an odd molecule and the situation in the crystal is very curious. The two insulin molecules in the asymmetric unit are very similar to one another, yet appear to differ in conformation in a number of details; it is comforting to see how closely the molecules described in the Chinese papers agree with Oxford findings. Earlier small differences between us are diminishing; it may well be that the final comparison will give us reliable estimates of the errors we must expect in general in placing atoms from X-ray data on proteins. The present Peking map at 1.80 Å resolution is the most accurate map available of the insulin electron density defined by experimental, isomorphous phase angles—and may well remain so. The calculations we are carrying out at Oxford, designed to show the electron density at 1.50 Å resolution, depend on the extension of the phases from 1.9 to 1.5 Å by various computing procedures, some very experimental. The total cross checking of our operations by the Peking analysis when we come to compare atomic coordinates should be very valuable.

Many of the comments made by the Chinese insulin research group on their findings are similar to ones we have made ourselves, but they often add illuminating details and occasionally recognise quite different features—for example, they realised that the dihedral angles of the glycine residues, B8, B20 and B23, were all in the region allowed for D residues. This led directly to experiments by the Shanghai chemists and biochemists who showed that B23 might be replaced by D-alanine in the insulin sequence with only partial loss of biological activity, whereas activity vanishes when B23 is replaced by L-alanine.

The second paper carries a stage further the study of structure–activity relations in the insulin field through direct measurements of the interaction of insulin and insulin analogues with receptor proteins in liver and fat cells. It, too, parallels and extends work in other laboratories.

Soluble receptor protein preparations were made by the method of Cuatrecasas, of fat cells by a new method avoiding collagenase. It was found that despentapeptide insulin had almost the same binding capacity as insulin, while deshexapeptide insulin and the corresponding molecule with B23 replaced by D-alanine had reduced, but definite, binding capacity. The results strengthen the view that the non-polar residues of the B chain surface, B12, B16, B24 and B25, are important in receptor binding and that the loop B20–B23 and perhaps also the salt bridge A21–B22 assist in stabilising the conformation in this region. There is a nice discussion in the first paper of the part that conformational variability may play in assisting membrane binding in this part of the insulin molecule.

It will be splendid if we can some day soon all meet and talk over the very interesting observations that are accumulating, East and West, on the structure and function of insulin.

Revising the Cainozoic polarity record

from D. H. Tarling

Now that the main plate tectonic band wagon has passed, the dust can be brushed off the marine magnetic anomalies that were so effective in getting it rolling. It is now possible to examine in more detail the record they provide of past geomagnetic changes and to refine the time scale of polarity changes.

The polarity time scale of Heirtzler *et al.* (*J. geophys. Res.*, **73**, 2119; 1968), based on anomalies recorded on profile V-20 SA in the South Atlantic, has stood up remarkably well. Profile V-20 SA was chosen as the longest available with no marked evidence of changes in the rate or direction of spreading, and the anomalies were dated by assuming that the observed spreading rate at the ridge axis—1.9 cm yr^{-1}—had remained unchanged for the past

80 Myr. The fact that modifications are only now becoming essential is remarkable, and the choice was, to say the least, inspired.

The first evidence of inaccuracies began to appear in 1971, as a result of discrepancies between the ages of fossil-dated sediments lying over igneous rocks which carried the magnetic anomalies and of observed differences of curvature on fracture zones in the South Atlantic (Le Pichon and Hayes, *J. geophys. Res.*, **76**, 6283; 1971), which indicated two-stage spreading (see Mascle and Sibuet, *Nature*, **252**, 464; 1974). At that time the evidence was taken mainly to confirm the available scale, as the differences were generally less than 5%, which were insignificant for the analyses then being carried out.

Since then, however, detailed studies of the anomaly patterns on fast spreading ridges, such as those described by Rea and Blakely on page 126 of this issue, combined with the polarity record in deep-sea sediments, such as that reviewed by Opdyke (*Rev. Geophys. Space Phys.*, **10**, 213; 1972), have provided a generally acceptable revision of polarity scales for the past 22 Myr. The older parts of the scale, however, are not so amenable to checking. Palaeontological dating of sediments lying immediately over igneous rocks which carry the anomaly pattern are uncertain because of the lack of precision in the radiometric dating of stratigraphic zonations of the Tertiary. This arises because most available dates are based on measurements of potassium and argon isotope ratios in glauconites within stratigraphically dated sediments. During the burial of sediments glauconite grows from illite, a mineral which often contains appreciable amounts of relic argon; and further burial may also be responsible for later loss of argon from the glauconite. Furthermore, most deep-sea drilling stopped on encountering the first igneous rocks, some of which are intrusive and therefore post-date the sediments and others of which could be more extensive, isolated flows that may have flowed out over the sedimentary deposits of a few million years. Nonetheless, on such studies, Sclater *et al.* (*Initial Reports of the Deep Sea Drilling Project*, **22**, 381; 1974) have suggested modifications to the ages of certain anomalies; in particular, anomaly 24, which is 60 Myr old on the original scale of Heirtzler *et al.*, is tentatively redated at 56.5 Myr.

Anomaly 24, the oldest found in the North Atlantic between Greenland and Europe, is one of the most critical anomalies (Vogt and Avery, *J. geophys. Res.*, **79**, 363; 1974). On the previous time scale, anomaly 24 predates the first eruptions of flood basalts in Greenland, the Faeroes and Britain, yet it seems reasonable to assume that such flood basalts would be released during tensional rifting immediately preceding continental separation, rather than after the separation has occurred. Molnar and Francheteau, on page 128 of this isssue, suggest one explanation for this discrepancy.

It is possible, they reason, that anomaly 24 is younger than previously believed and that similar arguments hold for the age of anomaly 32. Molnar and Francheteau are careful no to suggest a possible age for these anomalies because of the difficulties involved in obtaining precise radiometri ages for the flood basalts; the polaritie of these basalts indicate eruptions ove periods of less than 2 Myr, but th range of possible radiometric ages i large because of the presence of exces argon in some areas and the occurrence of argon loss in others. But careful segregations of the more reliabl radiometric ages, and the newly available palaeontological evidence for th ages of sediments associated with floo basalts in eastern Greenland (Soper *e al.*, *J. geol. Soc. Lond.*; in the press indicate a probable age for anomaly 24 of either ~~52 or~~ 55 Myr, depending on the accepted radiometric age of the Thanetian Stage (compare 52.5 My by Tarling, *Principles and Applications of Palaeomagnetism*, Chapma and Hall; 1971) on the basis of preliminary palaeontological dates fro deep-sea drill cores. This new evidenc now means that it should be possibl to produce a revised Cainozoic polarit time scale.

Such a revision will necessaril mean recalculation of the frequenc and duration of polarity changes re-evaluation of correlations between eustatic changes and rates of seafloo spreading, re-examination of the history of the breakup of the North Atlantic and other oceans, and so forth but the fact that age discrepancies o some 5% are now becoming so important really reflects the coming-of-age o seafloor spreading as an extremel precise technique.

Hepatitis vaccine: a note of caution

from Arie J. Zuckerman

Viral hepatitis type B is distributed worldwide and there is a particular need for a vaccine for groups which are at an increased risk of acquiring this infection. High risk groups include surgeons, physicians, dentists, nurses, laboratory workers and other health care personnel, haemodialysis patients and their attendants (*Nature*, **235**, 130; 1972), children of mothers who become infected during pregnancy (*Nature*, **249**, 105; 1974), drug addicts, patients and others residing in large institutions, and persons living in certain tropical areas where sanitation is poor. There is also the view, not shared by many, that military personnel should be immunised against hepatitis B.

The repeated failure to passage serially hepatitis B virus in tissue culture has hampered progress towards the development of a safe and effective vaccine. Attention has, therefore, been directed towards the use of other preparations for active immunisation against hepatitis B (*Nature*, **233**, 92; 1971). A known infective serum that contains hepatitis B virus was inactivated by heat and used as the immunogen by Krugman and associates (*J. infect. Dis.*, **122**, 432; 1970; *J. Am. med. Ass.*, **217**, 41; 1971; *New Engl. J. Med.*, **290**, 1331; 1974). The serum treated in this manner was not infective and it successfully prevented or modified hepatitis B in 69% of susceptible persons inoculated with the heated serum and challenged with the infective serum 4–8 months later. Diluted and heat-treated serum may be regarded as an 'inactivated vaccine' but it is a crude way of inducing immunity and it is unlikely to be accepted for general use. This work, however, laid the foundations for unconventional immunogens that could be used as vaccines, and indeed 200,000 doses of one such preparation are now available for preliminary trials in man.

Coat protein

Isolated viral coat protein challenges the body's immune mechanism in the same way as the whole infectious agent and the possibility of using purified spherical 20 nm hepatitis B surface antigen particles, which are free of detectable nucleic acid, seems attractive. Such experimental immunogens have been prepared and a limited number of susceptible chimpanzees have been inoculated with encouraging results. But a variable amount of host protein, and perhaps carbohydrate, may be complexed with hepatitis B viral protein in quantities apparently greater than those in most recognised viruses (Zuckerman and Howard, *Nature*, **246**, 445; 1973). These host proteins may include various pre-existing structures of the liver cell and may thus induce undesirable immunological reactions. Recently, it was reported that antigenic determinants related to human plasma proteins are constituents of hepatitis B surface antigen (Neurath *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2663; 1974). These determinants are apparently related to antigenic specificities on prealbumin, albumin, apolipoproteins C and D, and the gamma chain of immunoglobulin G. Other studies indicated that the larger polypeptides of purified surface antigen are probably adsorbed serum proteins which are necessary for the preservation of antigenic activity.

Host antigens

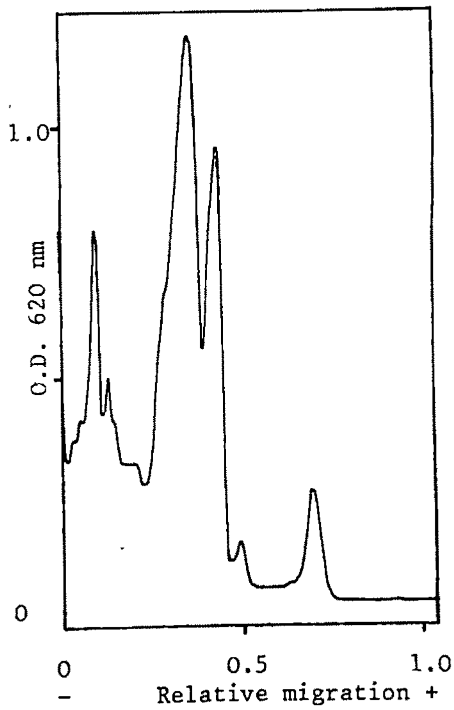
Demonstrable and significant levels of carbohydrate in purified fractions of the antigen have also been reported (Burrell *et al.*, *Nature new Biol.*, **243**, 260; 1973). The total carbohydrate content of purified 20–25 nm antigen particles is reported as 3.6 to 6.5%. Three glycoproteins were found as well as a glycolipid and three major phospholipids (Chairez *et al.*, *Intervirology*, **1**, 224; 1973 and **3**, 129; 1974; Steiner *et al.*, *J. Virol.*, **14**, 572; 1974). The carbohydrate may be necessary for maintaining the structure and functional integrity of the antigenic determinants or the carbohydrate itself may constitute a major antigenic determinant. The carbohydrate might have a novel haptenic specificity which is either virus-coded or virus-induced host cell-coded. Alternatively, the carbohydrate, and some lipoprotein components, might simply be derived from the host cell membranes as the mature virus particles are released. There may be some similarity between such a carbohydrate hapten, or the lipoprotein components, and those carbohydrate and lipoprotein antigens of normal cell surfaces, leading to a degree of tolerance because of a close antigen resemblance between hepatitis B surface antigen and 'self' antigens or alternatively initiating an autoimmune reaction.

Such an autoimmune reaction might be induced by hepatitis B infection because of a change in antigenicity of the hepatocyte cell membranes, due to alteration of existing antigens or the appearance of viral determinants. T lymphocytes responsive to such new antigen determinants could promote a B cell response to unaltered self antigens. The synthesis and release of the resulting autoantibody is subject in turn to control by suppressor T cells.

Liver-specific lipoprotein is a macrolipoprotein which is thought to be a normal constituent of the hepatocyte plasma membrane (Hopf *et al.*, *J. clin. exp Immun.*, **16**, 117; 1974). Williams and colleagues at King's College Hospital and at the London School of Hygiene and Tropical Medicine found evidence of cell-mediated sensitisation to hepatitis B surface antigen in all patients with acute hepatitis B. Transitory sensitisation to liver-specific lipoprotein was detected in many of the patients.

These findings are consistent with the hypothesis that a cell-mediated response to hepatitis B antigen, present early on at the onset of acute hepatitis, is the cause of acute liver damage by a cytotoxic effect on virus-infected cells. If the response to liver-specific lipoprotein persisted, it could be responsible for progression to chronic liver damage. It is also postulated that the progressive liver damage of active chronic hepatitis is due to an autoimmune reaction directed against an hepatocyte surface lipoprotein which is initiated in most cases by infection with hepatitis B virus. This was investigated by Lee and associates (*Br. med. J.*, **1**, 705; 1975) who observed evidence of cell-mediated immunity to hepatitis B surface antigen in 62% of patients with antigen-negative active chronic hepatitis, suggesting a high frequency of previous infection with hepatitis B virus. A cellular response to the antigen was found in the majority of hepatitis B antigen-positive patients. Evidence of sensitisation to liver-specific lipoprotein was found in more than half of the patients, with a similar frequency in the two groups. These results are consistent with the hypothesis that infection with hepatitis B virus is important in initiating the disease in many cases of active chronic hepatitis and that sensitisation to the liver cell membrane antigen is responsible for the perpetuation of the liver injury. Thomson *et al.* (*Nature*, **252**, 721; 1974) demonstrated the killing of isolated rabbit hepatocytes *in vitro* when incubated with lymphocytes from 20 out of 22 patients with untreated active chronic hepatitis. Blocking experiments strongly suggest that the cytotoxicity is due to an immunological reaction directed at a cell surface antigen.

All these observations indicate that immunological mechanisms and the presence of antibodies reacting with various tissue components may well be involved in the pathogenesis of liver damage. It may therefore be undesirable to employ preparations of hepatitis B surface antigen which contain host cell components for immunisation against this infection.



Profile of the polypeptides of purified hepatitis B surface antigen after SDS-polyacrylamide gel electrophoresis. The separated polypeptides were stained with Coomassie Brilliant Blue.

Subunits of the antigen, in the form of small polypeptides, on the other hand, offer an attractive proposition as immunogens. Such preparations would exclude genes of viral and cellular origin and could not be infectious. Several laboratories are investigating now the subunit structure of purified hepatitis B surface antigen by polyacrylamide gel electrophoresis and it has been shown that such subunits consist of polypeptides. It seems that the antigen contains between five and nine polypeptides ranging in molecular weight from approximately 15,000 to 120,000 (see figure). Work is currently in progress to determine if such preparations can be used as vaccines.

The primary sequence of the haptenic peptide of the surface antigen may also provide an approach for the development of a synthetic peptide, which when coupled to a macromolecular carrier could serve as a suitable immunogen. Once detailed data are available on the peptide and amino-acid composition of the antigen, it should be possible to define by animal immunisation the moiety responsible for the antigenic activity.

Immunisation against hepatitis B is now within reach but careful evaluation of the preparations in appropriate non-human primates is essential before small trials are undertaken in human subjects. These studies should be directed not only at the protective efficacy of such immunogens but they should include careful assessment of all their effects on the immune system. ☐

Actin and myosin in non-muscle cells

Secretion, motility and cell division

from Robert S. Adelstein

PROTEINS similar in molecular structure and biological properties to the muscle proteins actin and myosin have been identified in a large number of cells found in both vertebrates and invertebrates. Actin purified from non-muscle cells has the same monomeric molecular weight as skeletal muscle actin (42,000 daltons), contains a single residue of the unusual amino acid N^{τ}-methyl histidine and polymerises into filaments upon alteration of the ionic strength. The details of this polymerisation are not understood but it is thought that actin in non-muscle cells undergoes reversible polymerisation-depolymerisations unlike actin in muscle cells, which is thought to exist only as a filament.

Myosin from vertebrate non-muscle cells resembles muscle myosin (molecular weight 460,000) in being composed of heavy chains (200,000 daltons) and light chains (20,000 and 16,000 daltons in the case of fibroblasts, platelets and macrophages). Similar to muscle myosin, cytoplasmic myosin possesses an enzymic activity which catalyses the hydrolysis of ATP into ADP and inorganic phosphate. This ATPase activity is markedly enhanced at low ionic strength in the presence of Mg^{2+} by the addition of actin. It is noteworthy that cytoplasmic myosin interacts with muscle actin and *vice versa*.

Two major problems occupy the ever expanding number of workers attracted to this field: (1) the location of actin and myosin in non-muscle cells and (2) the function of these proteins (see also *Nature*, **253**; 399; 1975).

Three important cell processes have been postulated to involve cytoplasmic actin and myosin: secretion, cell division, and cell motility. It is important to note that all the evidence presented to date is at best circumstantial, in that no direct evidence for the role of actin and myosin in any of these processes has been reported.

In a recent article Burridge and Phillips (*Nature*, **254**, 526; 1975) presented evidence for the association of actin with the secretory granules of the adrenal medulla. These granules contain neurotransmitters which are released, following exocytosis into the blood stream. The authors succeeded in preparing membranes from partially lysed chromaffin granules which bound rabbit skeletal muscle actin (and very small quantities of chicken smooth muscle myosin). Binding was indicated by finding the contractile proteins and lysed membranes in the same fraction following sedimentation in a sucrose gradient.

The authors supplemented these studies with electron micrographs showing lysed chromaffin granule membranes associated with rabbit skeletal muscle actin filaments (the original photographs being superior in quality to those reproduced in *Nature*). In muscle cells actin is uniquely attached to a Z-line so that 'decoration' of the actin filament with a fragment of myosin, heavy meromyosin, leads to the formation of polarised 'arrowheads' pointing away from the Z-line attachment site. Unfortunately this uniform polarity is lacking in the preparation of membranes and actin filaments examined by these authors raising the possibility that at least some of the filaments might not be uniquely attached to the membrane.

Puszkin and Kochwa in a preliminary communication (*J. biol. Chem.*, **249**, 7711; 1974) presented data suggesting that contractile proteins are important in the release of neurotransmitters from brain tissue. They isolated a complex of actin and 'relaxing proteins' from rat brain synaptosomal membranes. The actin–'relaxing proteins' complex interacted with synaptic vesicles from brain, preloaded with ^{14}C glutamate and presumably containing brain myosin. The results of the interaction (in the presence of Ca^{2+}) were release of the labelled glutamate and activation of the Mg-ATPase activity of the myosin. The work is incomplete in that neither the relaxing proteins (presumably troponin) nor the myosin were positively identified. Moreover the release of ^{14}C-glutamate is assumed to correlate with the secretion of neurotransmitter.

Evidence that actin in HeLa cells (human tumour cells grown in tissue culture) might play a part during one phase of cell division was first proferred by Schroeder (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1688; 1973) who identified actin in the contractile ring of these cells. The identification was made by treating the cells with glycerine so they would be permeable to heavy meromyosin which interacted with actin filaments to form the unique arrowhead pattern alluded to above. Again, the evidence is circumstantial. The presence of actin implies, but does not prove, that it is functioning (perhaps with myosin?) to cleave the cells.

A role for actin in cell motility and phagocytosis was suggested by Boxer *et al.* (*New Engl. J. Med.*, **291**, 1093; 1974) who investigated polymorphonuclear leukocytes isolated from a patient suffering multiple infections. These cells failed to migrate normally an ingested particles at a slower rate than controls. Actin isolated from these cells could not polymerise to the same extent as actin from the white cells of normal controls. It must be stressed that only a single patient's cells were examined and that the process of actin polymerisation using non-muscle actin is not well understood at present. But the principle of using a cell showing a defect in its motile behaviour as a source for studying actin is important.

Future experiments in this field should be directed at: (1) complete characterisation of the various proteins and their interaction; (2) the *in situ* identification of the contractile proteins using antibodies that have been shown to be specific for either actin or myosin by a number of rigorous criteria; (3) the removal of a specific link (for example, Ca^{2+} ATP, troponin) in the contractile mechanism which results in changes that can be related to cell function; (4) further studies looking for specific defects in the contractile proteins of abnormally functioning cells; (5) identification of a specific inhibitor of actin–myosin interaction which can be used *in vivo* (as well as *in vitro*) to study changes in cell function. The list, of course, can be ex

A hundred years ago

Curious Phenomenon of Light

ROWING on Loch Lomond recently, above Luss, there were seen to the north-west, at an apparent distance of about 100 yards, two bright lines of prismatic light, 60° apart and on the level of the water. Their length seemed to equal the breadth of a rainbow. Their violet ends were towards each other, and were joined by a line of dull white light, to the middle of which the sun and the spectator were at right angles. Standing in the boat, the colour and brilliancy were lost, and only a diffuse white light was visible. The time was 10 A.M. The sun was hot, the sky cloudless, the air hazy and still, and the loch a mirror. This apparition fled before our approach for some minutes, till dispelled by a slight breeze, which rippled the water.

Luss WM. M'LAURIN

from *Nature*, **12**, 26; May 13, 1875

panded but perhaps in the near future we shall have direct evidence as to the exact role of cytoplasmic actin and myosin.

Cell movement

from Robert Shields

SINCE the discovery that non-muscle cells contain actin it has become almost an article of faith among cell biologists that cell movements are somehow generated by actin–myosin interactions. Unfortunately non-muscle cells do not present their actin–myosin complexes in the nicely structured array seen in striated muscle and although microfilaments have been shown to contain actin-like proteins quite how this was translated into cell movement has been obscure until recently.

Cellular locomotion is most conveniently followed in tissue culture where it has been clear for some time that only limited areas of the underside of the cell approach closely to the surface of the culture vessel. Electron microscopy of a section of a cell's leading lamella reveals that electron dense plaques form on the inside of the cell membrane where the cell comes closest to and probably adheres to the culture vessel. These points can be seen in scanning electron micrographs just behind the ruffling cell membrane (Revel *et al.*, *Expl Cell Res.*, **84**, 207; 1974).

Soon after the formation of the plaques bundles of microfilaments can be detected running obliquely from the plaques towards the rear of the cell (Abercrombie *et al.*, *Expl Cell Res.*, **67**, 359; 1971). This arrangement of adhesion plaques near the cell borders and microfilament bundles running from the plaque parallel to the long axis of the cell has been beautifully confirmed in scanning electron microscope studies of the replicas of the undersides of untransformed BHK cells (Revel and Wolken, *Expl Cell Res.*, **78**, 1; 1973).

Because of the abundance of these points of adhesion near the advancing edges of cells it was natural to assume that cell locomotion might result from pulling of the microfilament bundles on the points of adhesion to the substratum. Evidence to support this idea has recently emerged in a series of papers from a group at Cold Spring Harbor. What these workers did was to raise antibodies to purified actin or purified myosin (which is no mean feat) and use indirect immunofluorescence to detect binding of actin or myosin antibodies to fixed cells by fluorescence microscopy. Untransformed 3T3 cells showed bright staining with actin antibody along microfilament bundles which seemed to be close to the underside of the cell and orientated away from the cell periphery (Goldman *et al*, *Expl Cell Res.*, **90**, 333; 1975). Similar patterns of staining were seen with anti-myosin antibody and in addition both antibodies stained the cytoplasm diffusely.

The intense patterns of staining along the microfilament bundles and the diffuse pattern over the body of the cell probably correspond to the two extreme arrangements of microfilaments encountered: bundles and a three-dimensional matrix that underlies much of the cell surface. The experiments strongly suggest that the bundles of microfilaments attached to the adhesion plaques contain both actin and myosin-like proteins and that the attachment of these bundles of filaments to the adhesion plaques could provide a mechanism for cellular locomotion.

Examination of cell replicas by scanning electron microscopy revealed another interesting feature. The underside of BHK cells transformed with polyoma virus showed few bundles of microfilaments or obvious points of cell substratum attachment, whereas such features were frequent in untransformed BHK cells (Revel and Wolken, *ibid*). Recent cell fractionation studies have also suggested that virally transformed cells have less actin associated with their cell membrane than do their normal counterparts (Wickus *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 746; 1975). A series of indirect immunofluorescence experiments using actin antibody and rat embryo cells transformed with a newly isolated temperature sensitive mutant of SV40 virus showed what was happening. At the permissive temperature (where the cell exhibits its transformed phenotype) the brightly staining actin-containing bundles of microfilaments are almost completely absent but the diffuse pattern of cytoplasmic staining remains; however, after cultivation at the non-permissive temperature (where the normal cell phenotype is re-expressed) bundles of microfilaments, which stain with actin antibody, appear (Pollack *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 994; 1975). From these results it would seem that transformed cells may have some defect in their ability to organise microfilament bundles and adhesion plaques and this may account for their altered cell–substratum adhesion and altered locomotion. ☐

Isotope clue to end of glaciation

by John Gribbin

THE use of measurements of oxygen isotope ratios to provide a clue to past temperatures is now a familiar tool of climatologists. But Kenneth and Shackleton have given a new twist to the technique in a study reported in *Science* (**188**, 147; 1975). They interpret a large change in the isotope pattern in planktonic foraminferans from Gulf of Mexico sediments as arising from the influx of fresh water from the melting ice sheet at the end of the Wisconsin.

This certainly seems more plausible than trying to explain that particular feature in terms of a temperature change, since it would require an increase of 8 °C to 33 °C at a time (between 15,000 and 12,000 years ago) when, judging by other evidence, the Earth was not in a warm phase. The change in salinity caused by the influx of fresh water to the gulf would produce the same effect (if surface salinity fell by 10%), and the argument is strengthened by a comparison of shells of deep-water creatures with those that lived near the surface; the isotope ratios in the latter show five times the change found in the former.

The model also fits in well with present understanding of the geography of the ice sheet, which would have prevented a substantial easterly flow of meltwater until about 12,000 years ago, when the Hudson River system opened up; before that time, the chief escape route for meltwater would have been a southward flow through the Mississippi River system.

All this tells us little new about recent glaciation. The real interest, of course, is a possible technique for investigating the extent of earlier ice sheets. As Kennett and Shackleton summarise the work, "earlier phases almost certainly would have influenced salinities in the Gulf of Mexico and will provide information on the extent of earlier ice sheets, since it can be assumed that no substantial meltwaters would have entered the Gulf unless the southward extent of the ice sheet was greater than the latest Wisconsin limit about 11,200 years ago. Such an approach will be of particular value since little is known about earlier episodes of deglaciation, because succeeding glacial advances have redistributed the older deposits".

Carbon dioxide fluctuations

from Peter D. Moore

THE global movement of carbon between non-living and living matter is one of the many cyclic processes upon which man's industrial activities are having considerable effect. The combustion of fossil fuels accelerates one part of the 'natural' cycling of carbon; it replaces the respiratory activity of decomposer organisms, whose consumptive processes were impaired by anaerobic conditions during the accumulation of the organic detritus which is now coal, oil and gas. Industrial combustion, however, is almost certainly responsible for the observed rise in ambient atmospheric carbon dioxide levels over the past sixteen years in Hawaii. Baxter and Walton (*Proc. R. Soc.*, **A318**, 213; 1970) suggest an increase of 22% on pre-industrial levels of atmospheric CO_2. Evidently the increased output of carbon dioxide, resulting from increasing industrialisation, is not being fully compensated by CO_2 consumption in other global reservoirs.

The major potential carbon reservoirs which might be expected to increase consumption of atmospheric CO_2 as concentrations rise are the oceans and the living organic matter of the planet. It is obviously a matter of considerable urgency to develop predictive models of this equilibrating system in order to determine the likely outcome of man's industrial activity; several such models now exist (for example, Fairhall, *Nature*, **245**, 20; 1973; Broecker *et al.* in *Impingement of Man on the Oceans*, edit. by D. W. Hood; Wiley, 1971). Opinions differ as to the likely influence of the additional carbon load upon the chemistry of ocean water (see Whitfield, *Nature*, **247**, 523; 1974).

World biomass variations are not easily estimated. One might predict increases in photosynthetic production as a consequence of enhanced CO_2 levels, but one must set against this the possibilities of reduced productivity because of raised SO_2 and O_3 concentrations in the atmosphere of industrial countries and also the destruction by man of many naturally productive areas. The production : respiration ratio of the world's biota could be moving in either direction. Reiners (and several other contributors to *Brookhaven Symp. Biol.*, **25**; 1973) have drawn attention to the general lack of reliable data and the wide disparity among estimates of such carbon reservoirs as living biomass and detritus pools, let alone transfer rates. Attempts have also been made to use tree growth rings as indices of forest production (for example, Whittaker *et al.*, *Ecol. Monogr.*, **44**, 233; 1974) and Farmer and Baxter (*Nature*, **247**, 273; 1974) have used the isotope ratio of $^{14}C/^{12}C$ in efforts to identify the contribution of fossil carbon to the growth rings of trees in Britain.

In this issue of *Nature* (page 136) Hall, Ekdahl and Wartenburg use a method which has been applied in many smaller scale studies to estimate trends in the biotic metabolism of carbon in the Northern Hemisphere. Their idea is based upon the flux study concept as used for the determination of forest respiration by Woodwell and Dykeman (*Science*, **154**, 1031; 1966), but rather than diurnal changes, they use seasonal fluctuation in ambient CO_2 levels (values are corrected by subtraction of the estimated residuum of industrially derived CO_2 after partition with the oceans). Increasing CO_2 levels in autumn and winter provide a measure of the respiration of the hemispheric ecosystem and decreasing levels in spring and summer give a measure of net photosynthesis. Using the data from the Mauna Loa observatory, Hawaii, they calculate hemispheric net production and respiration for the sixteen years over which data are available and they find no significant change during this period. This suggests that either the total biomass of the hemisphere is unchanged, or any loss of productivity due to pollution or changing land use has been compensated by an increase in the productivity of the remainder. It is possible to argue about the best figures to use for industrial carbon output and the equilibration of this carbon with the oceans (the prime considerations in the adjustment of the Mauna Loa data) but the authors claim that the outcome of their calculations is relatively insensitive to possible errors in these data. Their plea for a more extensive worldwide monitoring of atmospheric CO_2 fluctuations as an aid, among other things, to the detection of any major variation in global carbon metabolism, should be supported by anyone with a sense of responsibility for the environment.

Statistics and climatic change

from Michael Kelly

GREAT advances are currently being made in the study of climatic change. This progress is attributable not only to greater academic and, in certain countries, governmental interest in the subject but also to the increasing use of powerful statistical tools in the analysis of climatic time series.

Post-glacial climatic change can be viewed as movement between atmospheric circulation states of predominantly strong, westerly airflow (leading to warming of higher latitudes in the sector or sectors affected, as occurred globally in the first half of the twentieth century) and those of predominantly weaker, more meridional airflow (leading to cooling). Characteristic time scales or periodicities can often be attached to variations in climate; this had led to many attempts to explain the observed persistence of a particular circulation state for periods of months to centuries and also the change to a different state at fairly regular intervals Climatic cycles may eventually prove of great value in climatic forecasting but until a physical reason for their continued existence is established, such forecasts can only be tentative.

The search for the causes of climatic change and climatic cycles is proceeding on two fronts: computer modelling of the atmospheric circulation and statistical analysis of climatic data. The potential of both of these approaches is great but, at present, most computer models are too coarse to differentiate between different circulation states experienced during the last millennia and it is the second, more traditional approach to the study of climatic change that has been greatly improved by the availability of new statistica methods and that is rapidly increasing understanding of climatic change.

A recent paper by Trenberth (*Q. J R. met. Soc.*, **101**, 55; 1975) illustrates the use of sophisticated data analysis in a study of ocean–atmosphere interaction in the region of New Zealand and Australia. Empirical orthogona function analysis was used to identify the major sea level pressure (SLP) anomaly patterns, that is, atmospheric circulation states, and the major sea surface temperature (SST) anomaly patterns for the period 1959–1972 Spectral and cross-spectral analysis o time series of the pattern coefficients then revealed relationships, both current and lag, between the SLP and SST anomaly patterns, associated with a quasi-biennial periodicity which ha been observed in many climatic parameters. These relationships enabled Trenberth to construct a sequence o SLP and SST anomaly charts for the two-year cycle, which showed that the SST anomalies were largely caused by oceanic advection resulting from the wind drag associated with the differen SLP patterns.

This research did not indicate tha feedback from ocean to atmosphere was of great importance in the region studied. But work in other areas, particularly the Pacific and North Atlantic has shown that anomalous sea temperature patterns often reinforce the atmosphere circulation states that produce them Although this positive feedback can explain the persistence o certain atmospheric circulation states it cannot explain the observed regularity of many climatic cycles. If this is to be explained in terms of ocean–atmosphere interaction, some form of complex negative feedback between ocean and atmosphere must be postulated.

Complete nuclear isobaric quintet

from P. E. Hodgson

IF nuclei of the same mass (isobars) are grouped together, it is found that their states can be conveniently classified using the isobaric spin vector **T**, analogous mathematically to ordinary spin. States of the same structure in different isobars have identical nuclear quantum numbers, including isobaric spin, and differ only in the third component T_3 of the isobaric spin vector. This quantum number T_3 is directly related to the excess of neutrons over protons, thus $T_3=\frac{1}{2}(N-Z)$.

For example, the ground states of ^{25}Mg (12 protons and 13 neutrons) and ^{25}Al (13 protons and 12 neutrons) have the same nuclear structure and $T=\frac{1}{2}$, but the former has $T_3=\frac{1}{2}$ and the latter $T_3=-\frac{1}{2}$. These states constitute an isobaric spin doublet. Some of the higher states of these nuclei have $T=3/2$, and then as before $T_3=\frac{1}{2}$ and $T_3=-\frac{1}{2}$ correspond to states in ^{25}Mg and ^{25}Al of the same structure, and in addition there are states of the same structure to be found in ^{25}Na (with $T_3=3/2$) and ^{25}Si (with $T_3=-3/2$). These four states comprise an isobaric spin quartet. The states of nuclei with an even number of nucleons can be similarly classified into isobaric singlets, triplets, quintets and so on.

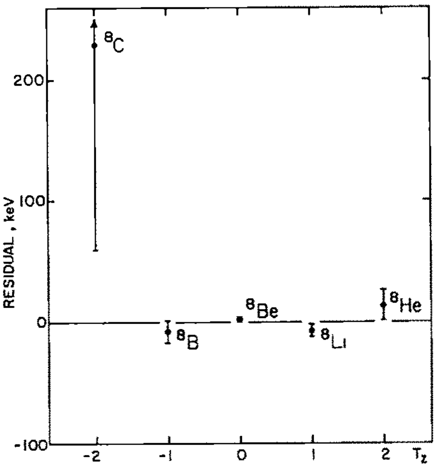
Analysis of the structure of these isobaric spin multiplets, as they are called, showed that for states of pure isobaric spin the mass excesses of nuclei in a particular multiplet are given by the simple quadratic expression

$$\Delta M=a+bT_3+cT_3^2$$

where a, b and c are constants. Multiplets with up to three members automatically satisfy this relation, which is therefore not useful, but higher multiplets provide a means of testing its accuracy. Thus if the masses of all four members of a quartet are known then any three of them determine a, b and c, and from these the mass of the fourth can be calculated and compared with the measured values.

Over the past few years several such quartets have been measured, and on the whole have confirmed the above formula to a high degree of precision with some small systematic discrepancies that can be explained by higher-order effects.

A more severe test of the formula is provided by higher multiplets, but this is difficult experimentally because the members of these multiplets with higher T_3 are usually states of highly unstable nuclei. Recently however a complete set of masses for the quintet in nuclei of mass 8 comprising the lowest $T=2$ states in ^{8}C, ^{8}B, ^{8}Be, ^{8}Li and ^{8}He has been obtained by Robertson and Chien at Michigan State and Goosman at Brookhaven (*Phys. Rev. Lett.*, **34**, 33; 1975) and these have been used to test the isobaric mass formula. If the deviations from the above formula are represented by extra terms dT_3^3, eT_3^4, and so on, then a quartet determines one of these terms and a quintet two.



Amounts by which individual masses exceed those given by a weighted fit of the quadratic isobaric mass equation to the masses of the $A=8$ quintet.

The five masses were first fitted by the above equation with the addition of the dT_3^3 and eT_3^4 terms, and it was found that $d=-18\pm14$ keV, and $e=13\pm7$ keV, indicating that e, but not d, is probably significantly different from zero. The residuals in a three parameter fit to the above formula are plotted in the figure, and the odd–even staggering corresponding to a non-zero value of e is apparent. It is clear that a more accurate value of the mass of ^{8}C is needed, and this would increase the sharpness of the test.

There are several effects that could cause deviations from the simple three-parameter equation. One of these, the isospin mixing of $T=0$ and $T=2$ states in ^{8}Be, is readily calculable, and gives $d=0$ and $e\simeq-1$ keV. This is consistent with the result of a fit to the measured masses when d is set equal to zero. Remaining small differences may be caused by increasing Coulomb repulsion in the neutron-deficient members of the quintet.

The results from the first complete quintet thus confirm the high accuracy of the simple isobaric mass formula, and indicate that the very small deviations can be accounted for by calculable higher order effects.

Does San Miguel threaten San Diego?

from Peter J Smith

THE San Miguel Fault in Baja California (Mexico) strikes roughly parallel to the other major faults in the San Andreas system and, like those other faults, presumably results from the north-westerly movement of the Pacific Plate with respect to the North American Plate. The detailed relationships between the San Miguel Fault and faults further to the north in the United States have not yet been established precisely, however, although Wiegand (*Bull. Ass. engng Geol.*, **7**, 107; 1970) and others have proposed that a shear zone which begins with the San Miguel Fault in the south-east extends right through to the Inglewood fault zone in the north (the site of the Long Beach earthquake of 1933). This suggested shear zone would also include the Rose Canyon fault zone which passes through San Diego and Tijuana.

If these connections are proved, it follows that the behaviour of the San Miguel fault zone is pertinent to the problem of seismic hazard in San Diego; and for this reason Reyes *et al.* (*Geophys. Res. Lett.*, **2**, 56; 1975) have, since 1970, been measuring the microearthquake activity at various sites in the vicinity of the fault. They found that the overall rate of microearthquake activity at the south-eastern end was about 100 events a day (December 1970–December 1973), although some individual rates rose to 600 and many were significantly higher than those observed along most of the San Andreas Fault in southern California. By contrast, the overall activity towards the more critical (for San Diego) north-western end of the San Miguel Fault was about 20 events a day, with individual rates of up to 70 a day.

The San Miguel fault zone is thus microseismically active. But what does it mean in terms of larger, potentially destructive earthquakes? The problem, as Reyes and his colleagues acknowledge, is that microearthquake activity is not necessarily indicative of long term seismic activity. For example, the 1857 Californian earthquake occurred along a section of the San Andreas Fault which is now 'locked' and exhibits very little microseismicity, although in other sections of the fault microearthquake rates are consistent with the seismicity observed over the past few decades. But although precise correlations cannot be made, the activity observed by Reyes *et al.* suggests that the seismic risk to San Diego and Tijuana from the north-western San Miguel fault zone should not be taken too lightly. □

Organ culture

from Michael Balls and Marjorie A. Monnickendam

The British Society for Cell Biology held a symposium on organ culture in biomedical research at the University of East Anglia on 8–11 April, 1975.

IN the opening session, Dame Honor Fell (University of Cambridge) outlined the historical development of organ culture, and stressed that, though some well-meaning organisations were currently advocating the use of organ and cell cultures to replace experiments on live animals, there were insuperable objections to this as a general policy. Although organ culture experiments gave useful information which could not be obtained from animal experiments, there were many factors operating *in vivo* which could not be satisfactorily mimicked *in vitro*.

Giselle Hodges (Imperial Cancer Research Fund, London) reviewed the range of organ culture techniques currently in use, and J. Simnett (University of Newcastle-upon-Tyne) stressed the importance of care in the handling and preparation of tissues while setting up cultures.

The usefulness of organ culture in morphogenetic studies was shown in papers on germinal tissues (R. Dubois, Institut d'Embryologie, Nogent-sur-Marne), the kidney (Y. Croisille, Nogent-sur-Marne), cartilage and bone (Sylvia Fitton Jackson, Strangeways Laboratory, Cambridge), nervous tissue (S. Aparicio, University of Leeds), and skin and its derivatives (P. Sengel, University of Grenoble; A. Melcher, University of Toronto). C. Cruickshank (MRC Skin Unit, Birmingham) reviewed tissue culture studies on cell differentiation and function in adult skin, and W. de Voogd van der Straaten (Rijks Universiteit, Utrecht) discussed the suitability for cell kinetics studies of the exfoliated chick tracheal epithelium *in vitro*.

A number of contributions demonstrated the value of organ culture techniques in endocrinology, and the problems which can arise. Isabel Forsyth and E. Jones (National Institute for Research in Dairying, Reading), and R. Dils (University of Nottingham) discussed the hormonal induction of milk fat synthesis and changes in enzyme activities and nucleic acid synthesis in cultured mammary glands. Margaret Ryle (Birmingham and Midlands Hospital for Women) outlined work on endocrine aspects of the ovary, comparing the results of short-term incubation and long-term culture, and drawing attention to the problems which arise because of the need to support the tissue and the use of sera in media. *In vitro* investigations on the developing and adult pars intermedia were described by P. Chatterjee (St Mary's Hospital Medical School, London), and Ilse Lasnitzki (Strangeways Laboratory) and D. Cannon (St Helier Hospital, Carshalton) discussed hormone effects and hormone metabolism in the prostate gland. The pig costal method for quantifying the activity of serum somatomedin (the active intermediary of growth hormone) was described by J. Williams (Institute of Child Health, London). Studies on the foetal rat pancreas concerned the control of insulin synthesis and release by islet beta cells in organ culture and in monolayer cell culture (A. Lambert, University of Louvain) and amylase production by acinar tissue maintained in chemically defined medium in circumfusion organ culture (L. Murrell, University of Tennessee).

J. Reynolds (Strangeways Laboratory) opened a more clinically oriented session with a discussion of *in vitro* methods for studying the role of hormones and vitamins in bone resorption. G. Easty (Institute for Cancer Research, London) then discussed the production of osteolytic substances by tumours. In a fascinating talk, he related *in vitro* and *in vivo* observations, showing that the release of osteolytic activity (due to prostaglandins and a high molecular weight component) by human breast tumours *in vitro* was inhibited by high doses of aspirin, and that, in an animal tumour model, bone lysis and hypercalcaemia (though not tumour growth) were inhibited by aspirin and related drugs. Etienne Wolff (Collège de France, Nogent-sur-Marne) described the methods he pioneered for the long-term maintenance of human tumours *in vitro*, and reviewed the results of investigations on tumour growth, growth inhibition, and the effects of ionising radiations. Dorothy Easty (Institute for Cancer Research) tackled the problem of quantifying tumour invasiveness by examining the ability of suspensions of tumour cells to penetrate various tubular and membranous tissues, and measuring the effects of various drugs on invasiveness. Elizabeth Defries (Imperial Cancer Research Fund) reviewed chemical carcinogenesis in organ culture, where the major problem is to determine the extent to which morphological changes *in vitro* are related to the ability of the tissues to form tumours *in vivo*. J. Masters (The Royal Infirmary, Edinburgh) critically reviewed an *in vitro* method for predicting the hormone sensitivity of human breast tumours. His cautious approach was backed up by J. Dickson (University of Newcastle-upon-Tyne), who pointed out that, although *in vitro* biochemical responses of human tumours to drugs and/or hyperthermia indicate the existence of sensitive populations of cells within tumours, because of the complexities of the host–tumour relationship, one could not assume that treatment effective on a tumour *in vitro* would be effective *in vivo*.

The effects of external agents on cultured tissues were described by several speakers. Sylvia Reed (MRC Common Cold Unit, Salisbury) discussed the growth of viruses and mycoplasmas in organ culture. The technique has proved helpful in a number of ways; for isolating and growing viruses which would not grow in permanent cell lines, for examining the role of local immunity by comparing virus growth in tissues from immune and non-immune animals, and for examining interactions between viruses and mycoplasmas in mixed infections. There was no correlation between the virulence of viruses *in vivo* and their cytopathic effects *in vitro*. Lynne Reid (Cardiothoracic Institute, London) discussed the synthesis and secretion of glycoproteins by human bronchial submucosal glands, comparing normal and hypertrophied glands, and considering the effects of various drugs on them. T. Rae (Strangeways Laboratory) described a method for using rat foetal knee joints in culture as a means of measuring the tolerance of tissues towards materials used for orthopaedic implants. D. Lucas (University of Manchester) reviewed work on the effects of X-irradiation on embryonic, normal adult, and neoplastic tissues.

Although most of the discussion in the symposium was concerned with mammalian and human tissues, the host institution contributed three papers which illustrated the potential value of long-term organ cultures of amphibian tissues. These were concerned with the control of renin production and release (R. Worley), activation and blockade of hepatic adrenoceptors (D. Brown), and the hepatotoxic effects of high concentrations of paracetamol (S. Gater).

Concluding the proceedings, L. Franks (Imperial Cancer Research Fund) said that this should be the last symposium concerned with organ culture methods, for, like electron microscopy, the technique had now come of age: organ culture should now be regarded as a standard method which could be applied to most biological problems. He concluded that this was largely due to Dame Honor's unfailing ability to give good advice, and to her success in passing on her belief in the importance of a critical appraisal of an apparently ordinary object looked at from an extraordinary angle.

review article

Sex control in silkworms

Vladimir Strunnikov*

The production of hybrid silkworms yielding a very high proportion of male larvae is of great importance to the silk industry, because of the very much higher yield of silk from males. Strains giving as many as 98% male offspring have now been developed from mutants with translocations of the X chromosome.

THE problem of sex control in the silkworm *Bombyx* is one of great practical and theoretical importance. Male silkworms are more viable and produce more silk than do females. The commercial breeding of exclusively male silkworms would mean producing 15–18% more silk than is now obtained from bisexual strains. Furthermore, the use of sex control methods would solve the technically complex problem of preparing interbreed hybrids, since there would be no need for the present labour-intensive and inaccurate methods of separation of females and males.

Female silkworms are heterogametic (XY) and the males homogametic (XX). (The X and Y chromosomes of lepidoptera are often known as Z and W respectively.)

A variety of environmental agents, irradiation and thermal shock for example, disturb the normal processes of germ cell maturation and fertilisation and form the basis for the experimental control of sex in silkworms. In this article I will review the application of 'chromosomal engineering' for the production of unisexual and 'autosexing' strains of *Bombyx mori.* Four techniques will be considered, two of which are already of commercial interest.

Artificial parthenogenesis

Artificial parthenogenesis was discovered in silkworms by Tikhomirov in 1886. In his experiments, as in those of many other investigators, parthenogenetic eggs developed abnormally, and only in rare cases hatched into larvae.

In 1940 Astaurov[1] developed a simple and very reliable method for obtaining parthenogenetic offspring. He dissected eggs from the ovarioles and, after ageing for 10–12 h, immersed them in a water bath at 46 °C for 18 min. After this treatment the eggs were kept in water at room temperature for 5–10 min. The activated eggs were then kept at 15–17 °C and 80% r.h. for 3 d, and then under normal conditions.

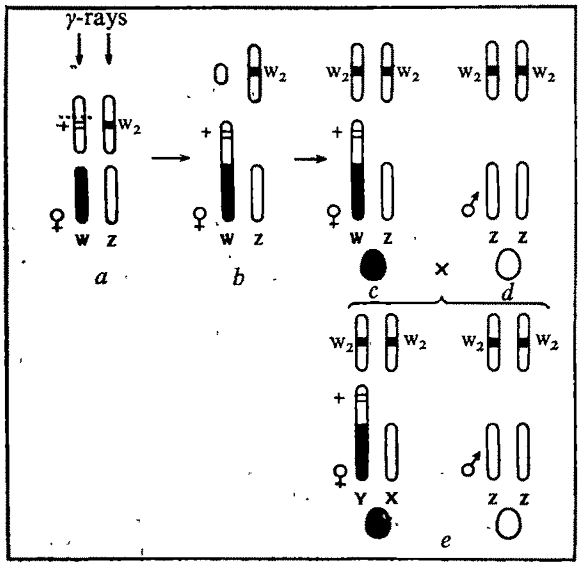
When layed the silkworm egg's nucleus is blocked at metaphase I. Normally fertilisation stimulates both the completion of the reductional first meiotic division and the subsequent equational second division. Heat treatment of unfertilised eggs apparently stimulates these maturation divisions and at the same time destroys the first division spindle.

The second meiotic division is normal and diploid pronuclei are formed. These pronuclei are all XY and hence all parthenogenetic individuals are female and identical in genotype to their mother. By selection Astaurov[2] has produced strains from which 95% of eggs will develop, after activation, by ameiotic parthenogenesis. About 98% of the larvae develop into adult moths—a survival rate better than that of most bisexual hybrid strains.

Spontaneous parthenogenesis in silkworms ending in hatching is very rare[3,4]. The mechanism of spontaneous parthenogenesis differs from the previously described induced ameiotic parthenogenesis since both meiotic divisions occur normally—diploidy being restored by the fusion of daughter cleavage nuclei. Thus spontaneous parthenogenesis is meiotic. Its frequency can be increased by various agents, such as low temperature (−5 to −17 °C), high temperature (44–50 °C) or exposure to CO_2[5,6]. These treatments also induce meiotic parthenogenesis in 95% of eggs, which reach the stage of the pigmentation of the serose, and only 2–3% of eggs are induced towards ameiotic parthenogenesis. With an increase of exposure doses the eggs are stimulated only towards ameiotic parthenogenesis.

After meiotic parthenogenesis two types of embryo are formed; XX and YY, both being completely homozygous. YY embryos are inviable. Presumably because of homozygosis

Fig. 1 The production and use of Y:10 translocation with w_2^+ for the production of a strain in which all male eggs are white and all female eggs coloured.



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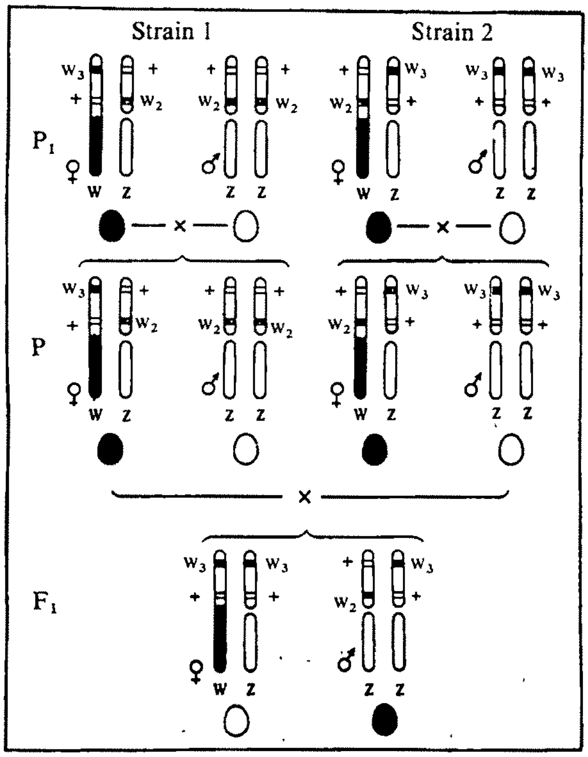


Fig. 2 Construction of two sex-marked strains using the Y:10 translocation and the linked egg colour mutants w$_2$ and w$_3$. Both strain 1 and strain 2 breed true and have coloured female and white male eggs. Their F$_1$ has coloured male and white female eggs.

for lethal or semilethal mutations only 8–9% of XX embryos hatch; they are, of course, all males. Because of the greater viability of these heterozygous females, compared with the homozygous males, the larval sex ratios are usually of the order of 93 males to 7 females.

Thus, depending on the type of activation technique used silkworms which are either entirely females (and heterozygous) or predominantly males (and homozygous) can be produced at will.

Dispermic androgenesis

Dispermic androgenesis in silkworms was discovered independently in Japan by Hasimoto[7] and in the Soviet Union by Astaurov[8]. As a result of heat treatment of eggs one hour after laying (for 1 h at 40 °C)[6] or of X-irradiation of oocytes and heat treatment after insemination (18 min at 46 °C)[8] the maturation divisions are so disturbed that no female pronuclei are formed. Since silkworms are polyspermic the opportunity then arises for two different male pronuclei to fuse and form a diploid zygote nucleus. Since the male silkworm is homogametic all individuals developing by androgenesis are male.

The irradiation of oocytes in the body of a female moth before copulation with the doses of 80–90 krad, after further heating, yielded twice or three times as many androgenous caterpillars as were obtained from non-irradiated but similarly heated eggs. The embryos formed after the fusion of an irradiated female pronucleus with a male pronucleus are unviable. That is why the irradiated oocytes give only androgenous male offsprings[9,10]. The timing of the subsequent heat treatment is critical—it should coincide precisely with prophase of the second division (70 min after laying at 23 °C). Under optimal experimental conditions as many as 20–30% of treated eggs may develop as androgenous males[11].

Double insemination of females by different males allows normally viable androgenous offspring to be produced following fusion of sperm pronuclei from different fathers[12].

Neither parthenogenesis nor dispermic androgenesis is yet a practical technique for producing a single sex in silkworms on a commercial scale. Two techniques that serve the same end and are commercially viable will now be described.

Development of sex-marked breeds

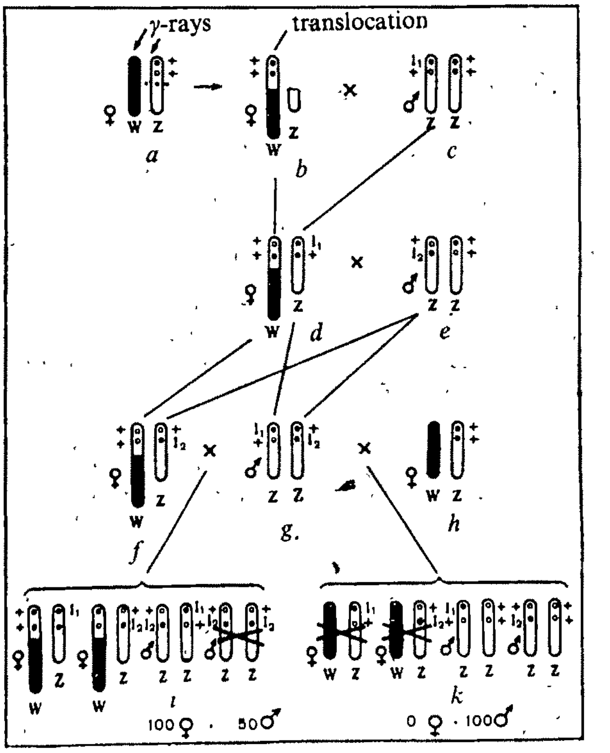
One of the first steps toward evolving methods of sex control on a commercial scale was the development of sex-marked silkworm breeds. At first one such breed, sex-marked in the egg stage, was obtained by Tazima[13] and later two breeds of this kind were evolved in the Soviet Union[14]. Although these breeds have never had a wide distribution in silkworm breeding, the methods used in making them will be described below to make it easier to understand the need for, and the essence of, more complicated methods for obtaining faultless sex-marked breeds.

On chromosome 10 of the silkworm there is a gene, w2, the wild type allele of which determines the formation within the developing embryo of a dark pigment giving the embryos their normal coloration. A recessive mutation at this locus blocks the formation of this serosal pigment and the eggs remain white.

An irradiation induced translocation has transferred the w_2^+ allele onto a Y chromosome (Fig. 1). Strains in which both the chromosomes 10 are structurally normal and the Y chromosome carries the translocation are viable, although somewhat reduced in weight, despite aneuploidy for the region of chromosome 10 distal to the translocation break point. If the chromosomes 10 are both marked with the recessive w$_2$ the translocation females will produce, on crossing to homozygous w$_2$ males, two types of egg—white and coloured—which will develop exclusively into males and females respectively.

Although a practical method for distinguishing male and female embryos, this technique suffers from the fact that the w$_2$ homozygous males have lowered viability. For this reason 'autosexing' strains have been developed which have dark female and white male eggs but which, when crossed, give dark male eggs and white female eggs[15].

Fig. 3 The construction and use of a balanced X-linked lethal stock by the use of an X:Y translocation which covers both lethal loci. The stock is balanced (bottom left) with a 2:1 sex ratio. On outcrossing only male progeny are viable (bottom right). See text.



Aneuploid segregants from the Y:10 translocation that carry the translocated Y:10 element and a single normal chromosome 10 are viable and fertile females. In strain 1 the translocated chromosome is marked with w_2^+ and w_3. w_3 is a recessive mutation also localised at chromosome 10 which also results in white eggs. It fully complements with w_2. The normal chromosome 10 of strain 1 females is marked with w_2 and w_3^+. The males are homozygous for w_2. Strain 2 carries the same translocation but now marked with w_2 and w_3^+ and with its normal chromosomes 10 marked with w_2^+ and w_3. Both strains breed true and have coloured female eggs and white male eggs. Yet when strain 1 females are crossed to strain 2 males all male eggs are wild type and all female eggs are white (Fig. 2).

The advantage of these strains over that previously described can be seen from a consideration of the logistics of silkworm breeding. During the production of pure breeds the females are more important than the males—in these strains the females have coloured eggs and full viability. But for silk production males are more important than females and, in the strain hybrid, it is they that have the wild type egg colour. The translocated females resume their normal weight with the restoration of the balance of the tenth autosomes.

Automatic photoelectric devices can sort out hybrid eggs according to colour, and hence sex, at a rate of 140 eggs^{-1}. This may seem high yet it is inadequate for commercial breeding which requires the separation of tens of billions of eggs. This method is only used for the preparation of hybrid stock, hybrid males for commercial breeding being isolated by a different genetic method.

Use of balanced sex-linked lethals

X-linked lethals have been used in two ways to produce offspring that are entirely male. Males heterozygous for an X-linked lethal give 2:1 male:female sex ratios when crossed to females. Males heterozygous for two different sex-linked lethals, will give no viable female progeny. The construction of such stocks is normally impossible but can be done, in silkworms, by either dispermic androgenesis or by the use of duplications of some regions of X-chromosomes.

I have used[16] dispermic androgenesis to construct males doubly heterozygous for X linked lethals. These males, when crossed to females, gave a sex ratio of 98.6 males to 1.4 females.

A more efficient method of producing males heterozygous for two different lethals on their X chromosomes has been developed using a translocation of the left end of the X chromosome on the Y chromosome. The translocated portion of the X carries the wild type alleles of both lethals and translocation bearing females are fertile despite the sex chromosome aneuploidy. The genetic programme for making the balanced lethal stock is shown in Fig. 3. Females of the balanced stock all carry the X:Y translocation and a normal X chromosome marked with either lethal 1 or lethal 2. The males are all heterozygous for both lethal 1 and lethal 2. This stock breeds true but when the males are outcrossed to wild type females nearly all female zygotes die as a result of the expression of either one of the lethals. The 0.1% or so females that survive do so as a consequence of recombination between the lethal loci.

The use, for silk production, of hybrid strains which produce entirely males will greatly benefit silk yield. Both the Y:10 and X:Y translocations described have been introduced into commercial breeds of the silkworm and are being used in commercial breeding in the Soviet Union.

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articles

Interaction between the daily heat balance and the tidal cycle

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In a sea area consisting partly of periodically drying tidal flats, the semidiurnal lunar tide, interacting with the daily variation of solar radiation, gives rise to both a beat in the amplitude of the daily water temperature cycle with a period of 14.76 d, and a variation of the daily mean temperature, with the same period. This is shown theoretically and by observation.

IN general, the frequency spectrum of temperature fluctuations at the Earth's surface is dominated by annual and daily frequencies and their higher harmonics. These peak frequencies of the spectrum originate from, and can be computed from, the variation of solar radiation at a given latitude[1]. The rest of the spectrum can be attributed to 'atmospheric noise' (such as varying cloudiness and advection of different air masses, for instance by depressions, interchanging heat with the Earth's surface).

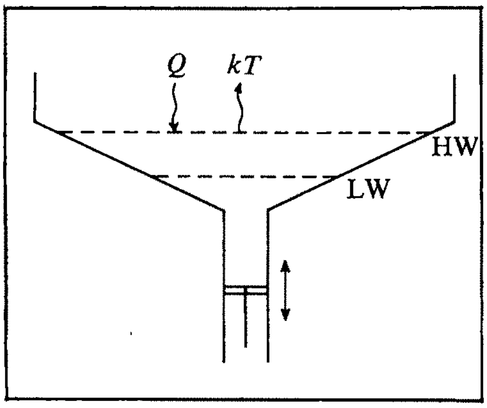


Fig. 1 Sketch of the model experiment described. HW, high water; LW, low water.

With respect to the sea surface the advection by periodic currents, such as those produced by the tides, may, in a non-homogeneous temperature field, also produce a peak in the frequency spectrum; the frequency being the same as that of the current involved if the temperature is measured in a frame of reference fixed to the sea floor. The latter fluctuations, however, disappear if the temperature is integrated over an area large as compared with the scale of the periodical currents.

This shows that the latter fluctuations arise from an internal redistribution of waterparcels but not from an external forcing agent such as the absorption of solar radiation and heat exchange with the atmosphere. It is, however, possible that in certain areas periodic (tidal) currents produce externally forced temperature fluctuations with frequencies that are sums or differences of the tidal and daily frequencies and their higher harmonics. This will be the case in those areas along the coast, where the effect of the tide is to produce a periodic flooding of tidal flats. This causes a periodical change in the surface area through which solar radiation penetrates and heat is exchanged with the atmosphere.

Knowledge of the heat balance and temperature cycle of such a tidal-flat sea is relevant to the biology of these areas; temperature is one of the most important physical factors in its ecosystem. Moreover, in planning power stations which will discharge heated effluents in areas of tidal flats, the special character of the heat balance should be taken into account.

Theoretical considerations

In a sea which covers tidal flats the tidal currents cause both a periodical translation of water masses and a periodical flooding of the tidal flats. In consequence, the area of interface between the sea water and the atmosphere will differ during different phases of the tide. At low tide a given volume of water will collect in deeper tidal channels and will thus have a smaller surface area than at high tide when part of the same volume will be spread out over the tidal flats.

To study the influence of such a periodically varying surface area on the heat balance of the water volume, we have devised a simplified model experiment which is assumed to represent the real situation in a tidal-flat sea.

The floor of a reservoir containing a volume V, of water of density ρ and specific heat c, slopes gently from the sides towards a hole in the middle (Fig. 1). The surface area of this volume of water can be varied by working a piston contained in a tube under the hole, up and down with a frequency, ω_2. The amplitude of the piston displacement is such that the water surface never reaches the sides of the reservoir and never becomes as small as the cross section of the hole. It is supposed that there is a varying absorption of (solar) radiation and a varying heat exchange between the water volume and the overlying air. Furthermore, it is assumed that the volume is well mixed in all directions so that it has at any moment a uniform temperature, T. The heat flux density, Q, through the water surface is then approximately given[2] by:

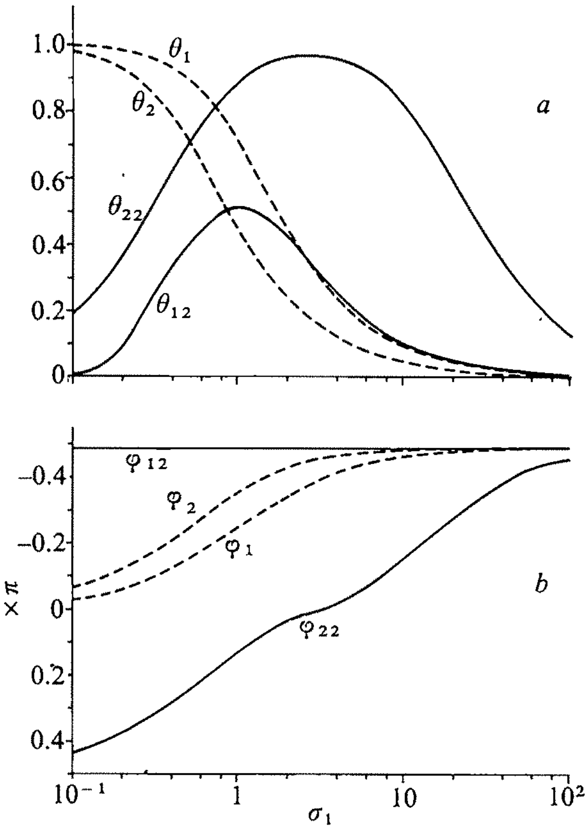
$$Q(t) = \sum_{n=-\infty}^{n=\infty} q_n \exp(in\omega_1 t) - kT(t), \quad q_n = q^*_{-n} \qquad (1)$$

where q_n is a (complex) amplitude of the Fourier series in $\omega_1 t$ (ω_1 is the daily frequency). The factor k is a bulk parameter[2,3] representing all the processes by which heat is exchanged with the atmosphere, such as long wave radiation, (turbulent) conduction, and evaporation. In general, k will be time dependent, but here it is assumed to be a constant. For $n \neq 0$ q_n contains both the daily variations in solar radiation and the effects of daily fluctuations in air temperature, humidity and wind velocity. We will, however, assume that these latter effects are of minor importance so that $q_n (n \neq 0)$ is only determined by the daily variation in solar radiation. Then, for a cloudless sky, q_n can be obtained easily for a given latitude and time[1]. For $t = 0$ at midnight, all q_n are real whereas for odd absolute values of n greater than 1 they are zero. For $n = 0$, q_0 represents the constant part of the heat income. It is, however, only approximately a constant because it varies during the course of the year and, on a smaller time scale, because of varying atmospheric conditions. It follows from equation (1) that the equilibrium daily mean temperature of the water mass is given by q_0/k (refs 2 and 3). We will assume that T represents the deviation of the water temperature from this equilibrium value.

Now, the rate of change in temperature of a volume, V, is given by:

$$\frac{dT}{dt} = \frac{Q(t).S(t)}{V\rho c} \qquad (2)$$

Fig. 2 *a*, Real, dimensionless temperature amplitudes; *b*, phase angles.



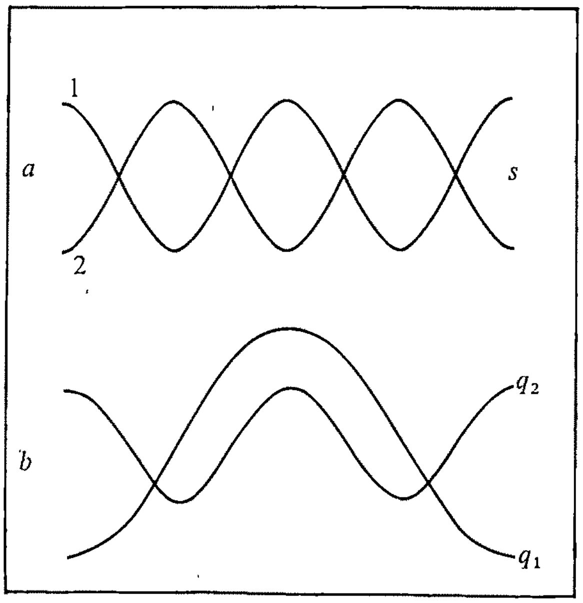


Fig. 3 *a*, Variation of surface area, *s*, during a day for two days separated by a time interval of seven days; *b*, variation during the day of the first and second harmonic constituents of solar radiation, q_1 and q_2.

Let $S(t)$ be given by:

$$S(t) = S_0[1+s_1 \exp(i\omega_2 t)+s_{-1}\exp(-i\omega_2 t] \qquad (3)$$

where $s_1 = s^*_{-1}$, and $2|s_1| < 1$

Without loss of generality $s_{1,-1}$ can be assumed to be real as long as $\omega_2 \neq n\omega_1$. We suppose ω_2 to represent the frequency of the semidiurnal lunar (M_2) tide.
Equations (1) – (3) give:

$$\frac{\mathrm{d}T}{\mathrm{d}t} = \left[\sum_{n=-\infty}^{n=\infty} q_n \exp(in\omega_1 t) - kT\right] \times$$

$$\times \left[1 + s_1 \exp(i\omega_2 t) + s_{-1} \exp(-i\omega_2 t)\right] / h\rho c \qquad (4)$$

where $h = V/S_0$ denotes the effective mean depth of volume V. Equation (4) is a first order non-homogeneous differential equation for T with a periodic coefficient of T and a driving term containing the frequencies $n\omega_1$ as well as their sums and differences with ω_2. We shall focus attention on the first and second harmonics of the daily frequency and their interaction with the tidal frequency, ω_2, as far as these interactions have frequencies smaller than ω_2. Thus, only the frequencies ω_1, $2\omega_1$, $\omega_2-\omega_1$, $2\omega_1-\omega_2$ are considered here, to which respectively the complex amplitudes T_1, T_2, T_{12} and T_{22} are ascribed. The latter can be obtained by inserting in equation (4):

$$T = T_1 \exp(i\omega_1 t) + T_2 \exp(2i\omega_1 t) + T_{12} \exp(i(\omega_2-\omega_1)t) + T_{22} \exp(i(2\omega_1-\omega_2)t) + \text{complex conjugates} \qquad (5)$$

and collecting terms with the same frequency. It is necessary to introduce several other quantities.

- $\tau = h\rho c/k$, a relaxation time[2,3] representing the thermal inertia of a water mass.
- Dimensionless frequencies $\sigma_1 = \omega_1\tau$ and $\sigma_2 = \omega_2\tau$.
- The frequency ratio $r = \omega_2/\omega_1$ $(1 < r < 2)$.
- Dimensionless real temperature amplitudes

$$\theta_1 = k|T_1/q_1|,\ \theta_2 = k|T_2/q_2|,\ \theta_{12} = k|T_{12}/s_1 q_1|,\ \theta_{22} = k|T_{22}/s_1 q_2|.$$

- Phase angles: $\varphi_1 = \arctan\,(Im[T_1]/Re[T_1])$ and φ_2, φ_{12} and φ_{22}. The solution for the θs and φs can be expressed in σ_1 and r and read, in first approximation:

$$\theta_1 = (1+\sigma_1^2)^{-\frac{1}{2}},\quad \varphi_1 = -\arctan\sigma_1$$

$$\theta_2 = (1+4\sigma_1^2)^{-\frac{1}{2}},\quad \varphi_2 = -\arctan 2\sigma_1$$

$$\theta_{12} = \sigma_1\,[\{1+\sigma_1^2\}\,\{1+\sigma_1^2\,(1-r)^2\}]^{-\frac{1}{2}}$$

$$\varphi_{12} = -\arctan\frac{1-\sigma_1^2\,(1-r)}{\sigma_1\,(2-r)}$$

$$\theta_{22} = 2\sigma_1\,[\{1+4_1^2\}\,\{1+\sigma_1^2\,(2-\mathrm{r})^2\}]^{-\frac{1}{2}}$$

$$\varphi_{22} = -\arctan\frac{1-2\,\sigma_1^2\,(2-r)}{\sigma_1\,(4-r)}$$

These functions are shown in Fig. 2*a* and *b* for $r = 1.932$, corresponding to the ratio of the tidal (M_2) frequency and the daily frequency. As k may vary[3] between 10 and 60 W m^{-2} K^{-1} and, for a tidal-flat sea, $0.1 < h < 10$ m is a representative interval, the range of interest for σ_1 will be $10^{-1} - 10^2$.

The components with the daily frequency and its second harmonic (θ_1, φ_1 and θ_2, φ_2) are the same as in the case of a water mass with a stationary surface area[2,3]. For $\sigma_1 \rightarrow 0$, $\theta_1 \rightarrow 1$ and $\theta_2 \rightarrow 1$, whereas $\varphi_1 \rightarrow 0$ and $\varphi_2 \rightarrow 0$, indicating that for increasing exchange factor k or decreasing depth or frequency the instantaneous water temperature approaches its instantaneous equilibrium temperature, defined by $Q(t)/k$, as it ought to be for a water mass with a very small thermal inertia.

The temperature fluctuations caused by the interaction of the daily heat balance and the tidal cycle have a maximum, however, if $\sigma_1 = 1.0$ and $\sigma_1 = 2.5$. In other words, if the relaxation time, τ, is of the same order of magnitude as the daily period. In contrast to the amplitudes for the daily frequency and its higher harmonics, the interaction amplitudes tend to zero for $\sigma_1 \rightarrow 0$. This should be so, as for a water mass which approaches at any moment its equilibrium temperature the variation of its surface area does not matter.

If ω_2 represents the M_2 tidal frequency, frequencies $\omega_2-\omega_1$ and $2\omega_1-\omega_2$ are, respectively, 6.780×10^{-6} and 4.925×10^{-6} rad s^{-1}. Therefore, one of the consequences of the interaction will be a long-period temperature variation with a frequency of $2\omega_1-\omega_2$ or a period of about 14.76 d. This will be observed as a variation of the daily mean water temperature.

The other interaction frequency $(\omega_2-\omega_1)$ corresponds to a period of about 25 h 42 min, somewhat larger than the daily period. As a consequence of the summation of trigonometric functions with slightly different periods, the sum of the temperature fluctuations with daily frequency and interaction frequency $(\omega_2-\omega_1)$ will be observed as a beat in the amplitude of the daily temperature cycle with a period of about 14.76 d. This can be seen as follows. If for $\exp(i(\omega_2-\omega_1)t)$ is written $\exp(-i(2\omega_1-\omega_2-\omega_1)t)$ we get for the daily temperature cycle:

$$\{T_1+T_{12}\exp[-i\,(2\omega_1-\omega_2)t]\}\exp(i\,\omega_1 t) + \text{complex conjugate}$$

Thus, the amplitude of the daily cycle reads:

$$2|T'_1| = 2\{T_1T^*_1+T_{12}T^*_{12}+T_1T^*_{12}\exp[i(2\omega_1-\omega_2)t] + T^*_1T^*_{12}\exp[-i\,(2\omega_1-\omega_2)\,t]\}^{\frac{1}{2}}$$

which is simply an amplitude varying with frequenc $2\omega_1-\omega_2$ or with a period of about 14.76 d.

The phenomenon of the interaction of the daily heat balance and the tidal cycle can be understood from Fig. 3. Here, for two days separated by a time interval of seven days, the variation of surface area (s) because of the M_2 tide is shown together with the first and second harmonic constituents of the solar radiation (q_1 and q_2). In case 1 the maximum and minimum of

Table 1 Real temperature amplitudes (°C)

h (cm)	$2\lvert T'_1\rvert$		$2\lvert T_2\rvert$	$2\lvert T_{22}\rvert$
	max	min		
10	3.62	3.15	1.06	0.33
50	1.39	1.13	0.25	0.40
100	0.73	0.56	0.13	0 39
250	0.37	0.24	0.08	0.29
500	0.18	0.14	0.03	0.19
1,000	0.09	0.07	0.02	0.10

q_1 coincide approximately with the maxima of the water surface, whereas in case 2 they occur at its minima. The consequence is a variation in the amplitude of the daily temperature cycle with a period of 14.76 d. As for the second harmonic, q_2, the maxima coincide with the maxima of s in case 1 and with the minima of s in case 2. Thus, in the first case there is a daily mean input of energy from this harmonic constituent whereas in the second case there is a daily mean output of energy. This causes a fluctuation of the daily mean temperature with a period of 14.76 d too.

In order to illustrate the theory we give a numerical example (Table 1) showing the values of the different real amplitudes for different depths of a tidal-flat sea. The values chosen for the parameters are: $k = 50 \text{ W m}^{-2} \text{ K}^{-1}$, $2|q_1| = 3\pi\ |q_2|/2 = 200 \text{ W m}^{-2}$, $2|s_1| = \frac{1}{2}$.

The interaction effects have a magnitude (Table 1) which is certainly sufficient to be detected with relatively simple techniques of measurement and which are, possibly, not without interest for ecological problems.

Of course, the interaction of the M_2 tide with the semidiurnal solar tide (S_2) does give rise to a spring–neap tidal cycle in the tidal range, which has the same period as the variation of the daily mean temperature and of the daily amplitude, explained here by the interaction of M_2 tide and solar radiation. Our explanation remains valid if an S_2 tide is present in the variation of surface area. In that case, the S_2 tide produces an apparent change in amplitude and phase of the temperature variations with daily frequency and higher harmonics. The existence of an M_2 tide interacting with solar radiation remains necessary, however, to explain the appearance of the 'new' frequencies $\omega_2-\omega_1$ and $2\omega_1-\omega_2$, and, consequently of the varying daily mean temperature and amplitude.

Real situation

Until now, there have been no systematic surveys of temperature fluctuations in tidal-flat seas. We have made extensive measurements in the Dutch Wadden Sea, which is part of an estuarine inshore area along the Dutch, German and Danish coasts, separated from the North Sea by a chain of islands. An important part of the area called 'Wadden', emerges at low tide as muddy sand flats. A typical part of this tidal-flat sea is the Mok bay, situated at the southern tip of Texel Island (Fig. 4). A tidal channel connects the bay with the western part of the Wadden Sea. Through this channel water which covers the tidal flats flows in and out with the tide. The mean volume of the bay is about 10^6 m^3; the area of the tidal flats, including the tidal channel, amounts to 8×10^5 m^2. The tide in this area is dominated by the M_2 tidal cycle. There is also an obvious spring–neap tidal cycle. The latter, however, has only a minor effect on the fluctuation of the surface area as, because of the geometry of the bay, high tide surface area and low tide surface area are approximately the same at spring tide and at neap tide.

In the tidal channel (Fig. 4b) the temperature was recorded at three different depths, at intervals of 5 min. For the purpose of this study, records taken at intervals of 15 min were sufficient. On that basis, the daily mean temperature was calculated. At one of the tidal flats all of the relevant meteorological parameters were recorded; these included net radiation, air temperature, humidity and wind velocity. The accuracy of water temperature measurements was checked with reversing thermometers, and seemed to be 0.1 °C. Measurements were carried out over three periods: April 21–May 5, 1972; August 22–September 21, 1973 and June 21–August 29, 1974. Thus, about 110 days of records were available for analysis As is to be expected in this area, daily mean values and daily amplitudes of water temperature varied greatly. To demonstrate that the records contain periodicities induced by the tide, as predicted by theory, 6 periods, each covering 15 d, were considered.

To separate short-term variations of the daily mean water temperature, including tidal effects, from background, long term fluctuations, such as increases or decreases of water temperature caused by seasonal heating or cooling, the daily mean difference between the temperature of the channel and that of the adjacent North Sea was calculated. For the latter, water temperature measurements taken from the lightship Texel (Fig. 4), positioned about 20 miles from Mok Bay, were used. These are thought to be influenced by long term meteorological processes, but not by the effects of the tidal flats.

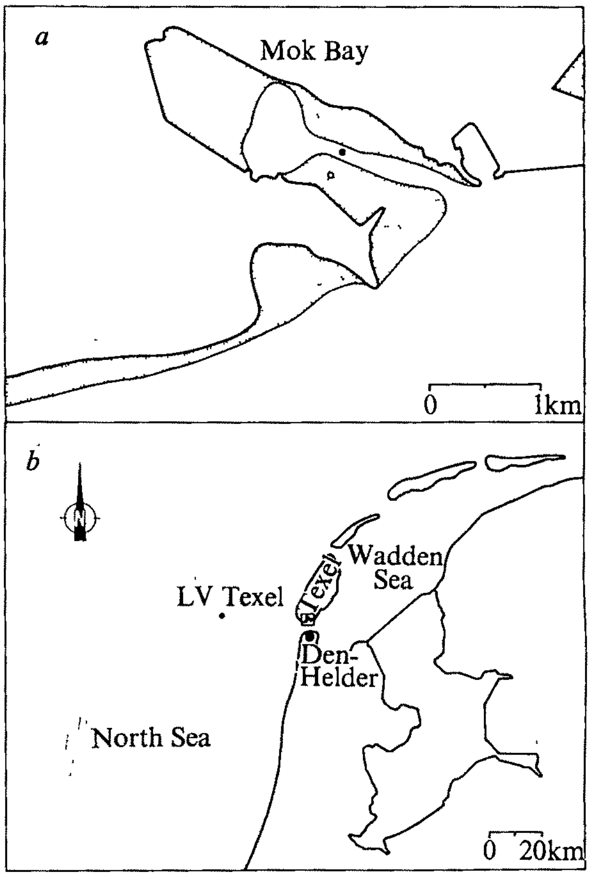
Next, for each of the six periods, a smoothed curve of the temperature differences was obtained using the formula: $\bar{X}_i = \frac{1}{4}(X_{i-1}+2X_i+X_{i+1})$, where $\bar{X}_i$ is the smoothed value for the ith day and X_{i-1}, X_i, X_{i+1} are daily mean values.

Then, for each of the six periods a straight line was drawn through the smoothed values by least-squares fit, to account for any trend during the period of 15 d. For each day in a particular period the deviation from this line was taken.

Finally, the last values were normalised for each period by dividing through the mean of the absolute values of the positive and negative extreme deviation from the least-squares-fit line.

After normalising the six periods, an average curve for each 15-d period was constructed in such a way that days having high tides at approximately the same time as one another occupied the same place in the sequence. The resulting curve, dimensionless by normalising, was transformed back to daily deviations in °C, from the period mean.

Fig. 4 Situation of the Mok Bay on large (a) and small (b) scale. The former shows the points of registration of meteorological parameters (○) and water temperature (●).



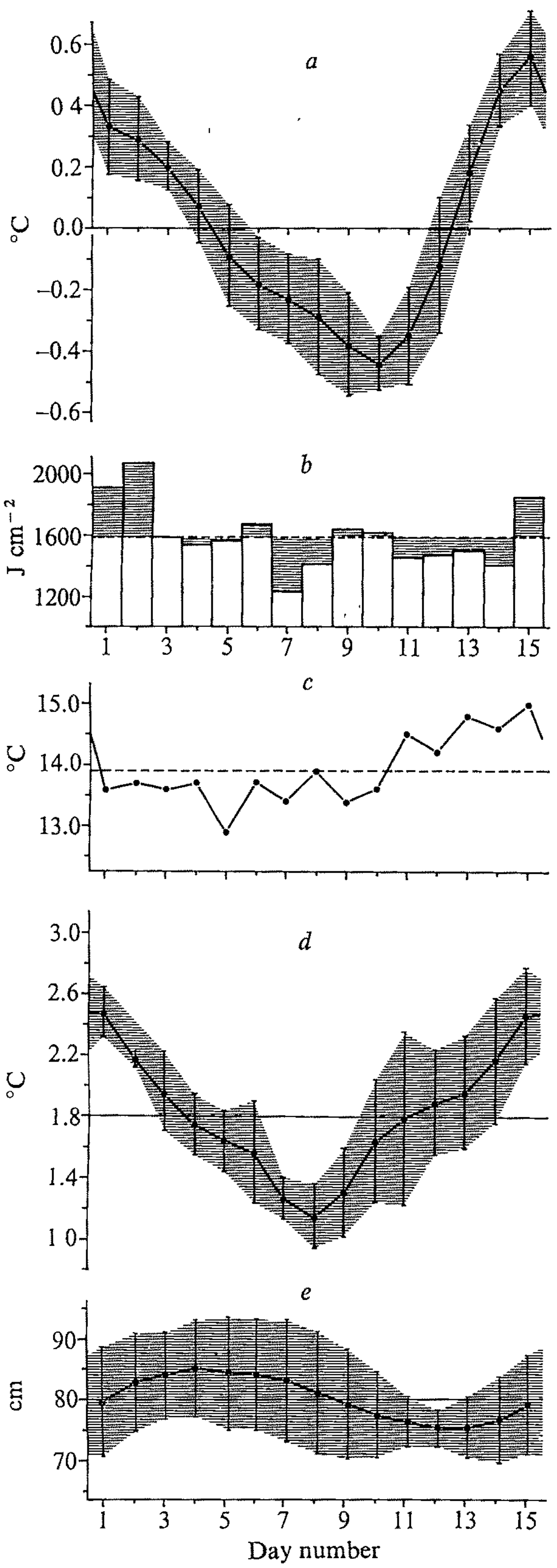


Fig. 5 *a*, Average curve of the deviation of daily mean water temperature of Mok Bay from its mean value over a 15-d period, showing also standard-deviation bars; *b*, average variation of global radiation over a sequence of 15 days; *c*, average curve of the daily mean air temperature; *d*, average curve of the difference between daily maximum and minimum water temperatures of the Mok Bay, showing also standard-deviation bars; *e*, average curve of high tide level in the Mok Bay over a sequence of 15 days; bars denoting the standard deviations.

The result is shown in Fig. 5*a*. There is a significant difference between the temperature deviation curve and any straight line and the curve agrees well with the predicted periodicity of the daily mean water temperature in a tidal-flat area.

To show that this result is not caused by an accidental variation of the energy input (q_o), having the same period, average values of global radiation, which constitutes the most important part of q_o, were calculated. For this we have taken values measured at Den Helder (Fig. 4*a*) by the Royal Netherlands Meteorological Institute. The mean global radiation for the 90 d used in the analysis is 1,585 J cm^{-2} d^{-1}. The average deviation for each day of the 15-d periods is shown in Fig. 5*b*. It is clear that there is no correlation between the variations in global radiation and the curve of Fig. 5*a*. For the same purpose this procedure was carried out for the air temperature. The mean air temperature for the 90 d, observed at land station Den Helder, supposed not to be influenced by any tidal effect, was 13.9 °C. The air temperature curve averaged over six periods is drawn in Fig. 5*c*. As for the global radiation, there is no apparent correlation between the average daily air temperatures and the curve of Fig. 5*a*.

The asymmetrical behaviour of the curve of Fig. 5*a* could be explained by the fact that, in this area, the moment of high tide itself shifts in an asymmetrical way during a 15-d period. Of course, part of the asymmetry may be caused by a variation in the atmospheric background, which has not been removed by averaging over only six periods.

As predicted theoretically, the daily amplitude varies with a period of about 15 d as well. As the maximum and minimum water temperatures are strongly dependent on weather conditions, the procedure used here to calculate the daily mean water temperature was used, without the first step and with a more extensive smoothing formula[4]:

$$\bar{X}_t = \tfrac{1}{35}[17X_t + 12(X_{t+1} + X_{t-1}) - 3(X_{t+2} + X_{t-2})]$$

To obtain the daily maximum and minimum water temperatures the mean values of the 5 highest and of the 5 lowest values of the daily records were taken (Fig. 5*d*). From this figure it may be concluded that the daily amplitude shows a periodic behaviour in agreement with theoretical prediction.

There is no need to regard the spring–neap tidal cycle as responsible for the results presented here, rather than the interaction of the M_2 tide and solar radiation. To illustrate this, we have shown (Fig. 5*e*) the average curve of the high tide level in the Mok Bay during a 15-d sequence. There is indeed a slight spring neap tidal cycle visible. Note, however, the large standard deviations which are caused partly by influences on water level other than those of the astronomical tides. The figure demonstrates, at least, that the maximum of the daily amplitude does not occur at spring tide and that the minimum does not coincide with neap tide. The same applies to the comparison of Fig. 5*a* with Fig. 5*e*.

The variation of the daily mean water temperature is about 0.9 °C, whereas the daily amplitude varies from 0.6 to 1.2 °C. These values are not in agreement with Table 1 if the mean depth is assumed to be about 100 cm. The numerical disagreement is probably caused by the great difference in geometry between the idealised theoretical model and the complicated tidal-flat area of the Mok Bay. Moreover, in theory a well mixed basin was assumed, whereas in reality temperature distribution in the Mok Bay is not uniform. Consequently, advection by tidal currents will affect the temperature variation, influencing especially the daily amplitude. None the less, it may be stated that a qualitative agreement exists between theory and experiment.

Received October 29, 1974, accepted March 10, 1975.

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Long-range transport of photochemical ozone in north-western Europe

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Measurements of the concentrations of ozone and trichlorofluoromethane in the atmosphere over various parts of the southern British Isles provides evidence to suggest that photochemical ozone and the pollutants from which it is formed may be transported into the region from their origins in continental Europe. It seems likely, therefore, that even isolated areas, away from centres of industrialisation, may suffer the effects of this pollution.

THE concentration of ozone in air at ground level increases during sunny, anticyclonic weather[1-3]. The maximum hourly concentrations of 0.11–0.12 p.p.m. are 2–3 times as high as expected in unpolluted air near to the ground. This suggests that photochemical ozone is widespread and is not confined to localities in which pollutants such as oxides of nitrogen and hydrocarbons are emitted.

In 1973 the ozone concentration was measured at three widely spaced locations on an E–W line across southern England and Ireland. The sites (Table 1) were in rural areas away from large, local sources of pollution and also away from forested areas and other sources of natural hydrocarbons. The most remote site, at Adrigole, was chosen in the expectation that it would provide a measure of the 'background' level of ozone concentration for the maritime regions of north-western Europe. Meteorological data were obtained from nearby meteorological stations (Table 1). In addition, local meteorological conditions at Harwell were recorded on an automatic weather station at the sampling site.

The ozone concentration was measured continuously using ethylene-chemiluminescence monitors. Ambient air was sampled at a height of 2 m above ground through polytetrafluorethylene tubing. A filter was used to prevent the entry of particulate matter into the detector and flow-control systems. The detectors were calibrated using a constant ozone source standardised with neutral-buffered, potassium iodide solution[4]. Regular checks on the calibration of the instruments at Harwell and Sibton revealed no significant drift in the zero or full-scale settings over the period of measurement. Routine calibration checks on the Adrigole instrument could not be made. The net change in sensitivity was, however, less than 10% over 5 months. Any zero drift was corrected by automatically recording the photo-multiplier dark current once every hour.

The concentrations of halogenated hydrocarbons as well as of ozone were measured at Adrigole. Air samples were taken twice daily, at 0500 and 1400, on an automatic gas chromatograph equipped with an electron capture detector operating in the coulometric mode[5]. The chromatograms were recorded on a strip chart from which absolute concentrations of CCl_3F and CCl_4 were determined at a later date. Similar measurements were made at Bowerchalke, Wiltshire (90 km south-west of Harwell) and using those results in conjunction with the Adrigole measurements we obtained information on the history of air masses over the southern British Isles at any given time. The trajectories of air masses were computed backwards in time for Adrigole and Sibton. These air trajectories were based on mean winds, computed for the layer 300–2,000 m above the average terrain using a Northern Hemisphere radio sonde observation data set. Each wind vector in the area around the point of interest was weighted by $1/R^2$ where R is the distance between the observing station and the point of interest. A 'central tendency' extrapolation scheme was used to trace the 'air' back in time.

Table 1 Latitudes and longitudes of sampling locations

Sampling location	Position	Meteorological stations
Adrigole, County Cork, Ireland	51°40′N 9°45′W	Valencia
Harwell, Oxfordshire, England	51°35′N 1°19′W	Heathrow
Sibton, Suffolk, England	52°03′N 1°05′E	Gorleston

Trajectories for all four synoptic times (0000, 0600, 1200, and 1800 GMT) were constructed, but only those for midday (1200 GMT) are considered here. The trajectories for Adrigole were carried backwards in time for a maximum of 5 d (120 h) which usually complied with the prescribed space limitations for wind vector interpolation.

At Sibton, it was possible to estimate the trajectories up to a maximum of 10 d (240 h) before the arrival of the 'air'.

Results and discussion of daily measurements

The maximum hourly mean ozone concentrations at th observation stations during each day are shown in Fig. 1. Th concentrations of CCl_3F at Adrigole and Bowerchalke are als shown. CCl_3F is an inert anthropogenic gas, and its concentra tion gives an index of pollutants in an air mass.

Ozone. Table 2 gives the frequency distribution of maximun hourly mean concentration of ozone. The range (0.03–0.1 p.p.m.) was similar at all three sites and, surprisingly, th number of days on which the US standard[3] of 0.08 p.p.m. w exceeded at Adrigole (17 days) was similar to that at Harwe

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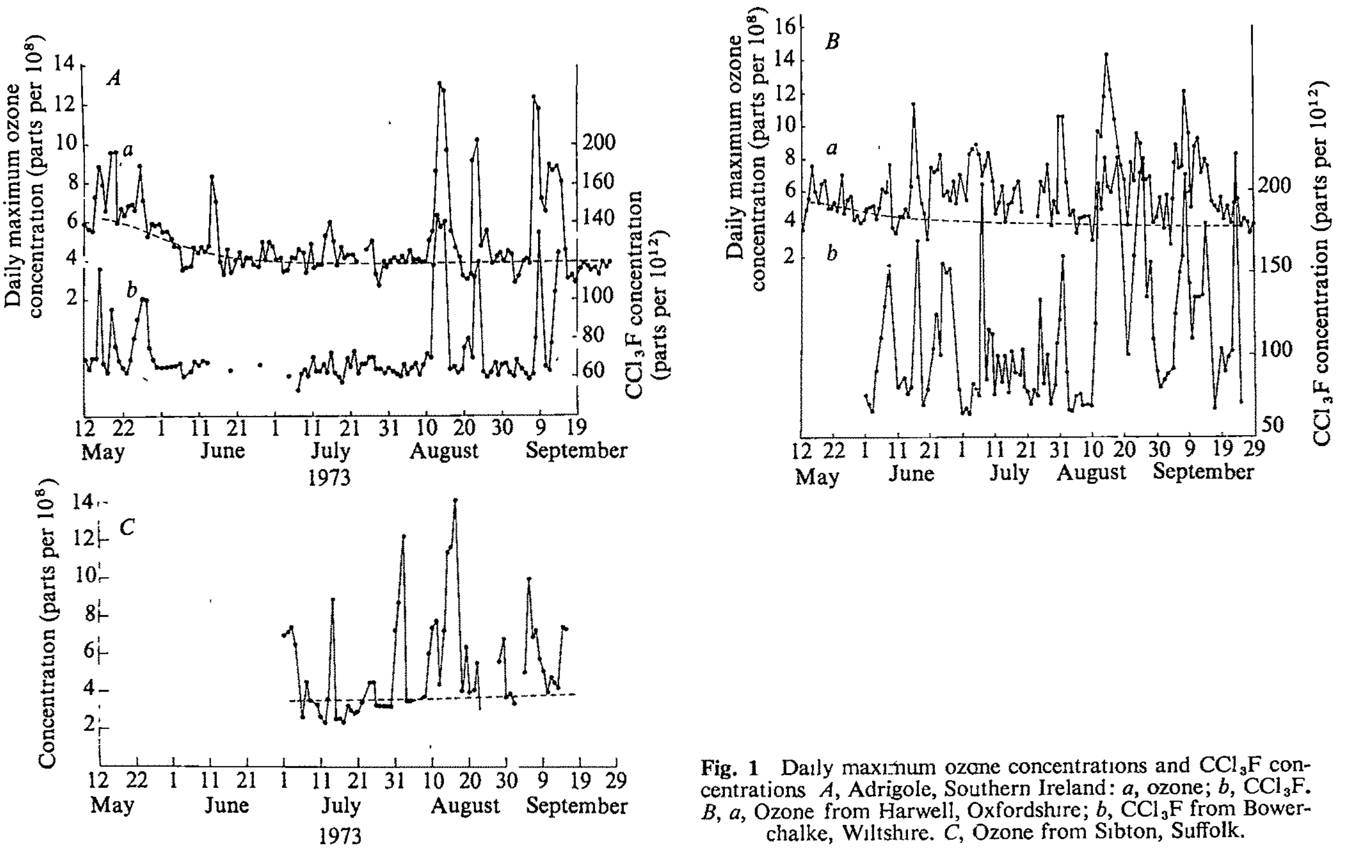

Fig. 1 Daily maximum ozone concentrations and CCl_3F concentrations *A*, Adrigole, Southern Ireland: *a*, ozone; *b*, CCl_3F. *B*, *a*, Ozone from Harwell, Oxfordshire; *b*, CCl_3F from Bowerchalke, Wiltshire. *C*, Ozone from Sibton, Suffolk.

(23 days) although Adrigole is much further from sources of the precursor pollutants than Harwell.

Incidents of elevated ozone concentration superimposed on a 'background' level (Fig. 1) are clearly indicated at each site. We believe this 'background' level represents the natural ozone content of the lower troposphere over north-western Europe.

The mean background concentration at Adrigole was about 0.04 p.p.m. compared with 0.03 p.p.m. at Mauna Loa and 0.02 p.p.m. at Point Barrow, Alaska (both in September, 1973).

The pattern of the incidents, particularly during August and September was similar at each of the three sites.

Fluorocarbons. The general pattern of the fluorocarbon concentrations was very similar to the maximum hourly mean ozone concentration data — values varied in southern England but stayed near background level for longer periods at Adrigole. The background concentration of CCl_3F at Adrigole was 68 parts per 10^{12} by volume (calculations were based on concentrations observed when the air masses were coming from the Atlantic). In polluted air, concentrations of up to 370 parts per 10^{12} by volume were observed. Figure 1 shows clearly that ozone and CCl_3F were advected in polluted air over south-western Ireland. Comparisons of ozone concentrations at Harwell with CCl_3F concentrations at Bowerchalke shows the same thing.

The correlation coefficient between CCl_3F and O_3 at Adrigole was 0.74, based on measurements taken at 1400 GMT May–September, 1973. The higher values ($CCl_3F > 100$ parts per 10^{12} by volume; $O_3 > 0.1$ p.p.m.) were deleted and the correlation recomputed, resulting in a reduced correlation coefficient of 0.49.

We conclude that a significant portion of the ground-level ozone in southern England during the summer is anthropogenic in origin and that in suitable meteorological conditions ozone and/or its precursors can be transported over distances of the order of 100–1,000 km, to give appreciable levels of pollution in locations far from the source.

Diurnal variation in ozone concentration

Figure 2 shows the diurnal variation of ozone at all three sites during August 11–18, 1973. Figure 3 shows the frequency distributions of the daily maxima as a function of time of day over the whole period of measurements at Harwell and Adrigole. At Harwell and also at Sibton, there were consistent daytime maxima and nocturnal minima. There was no marked diurnal variation at Adrigole. On several occasions maxima occurred during the night.

At inland stations shallow nocturnal inversions are common, particularly during anticyclonic weather in summer. Ozone is destroyed at the Earth's surface, and may also be removed by gas-phase reactions with other pollutants. Below an inversion, the downwards diffusion of ozone from above is inhibited, and thus the concentration can be expected to fall. During the day, convection is strong, more ozone is brought down, and the concentration rises. Adrigole is on a peninsula and is only

Table 2 Frequency distribution of maximum hourly average ozone concentration for the period May 12, 1973 to September 29, 1973

Maximum O_3 concentrations (parts per 10^8)	0–4.9	5.0–7.9	8.0–10.9	11.0 or greater	Data missing
		Number of days			
Adrigole	85	33	13	4	6
Harwell	51	60	18	5	7
Sibton (July 1–September 29 only)	47	21	4	4	15

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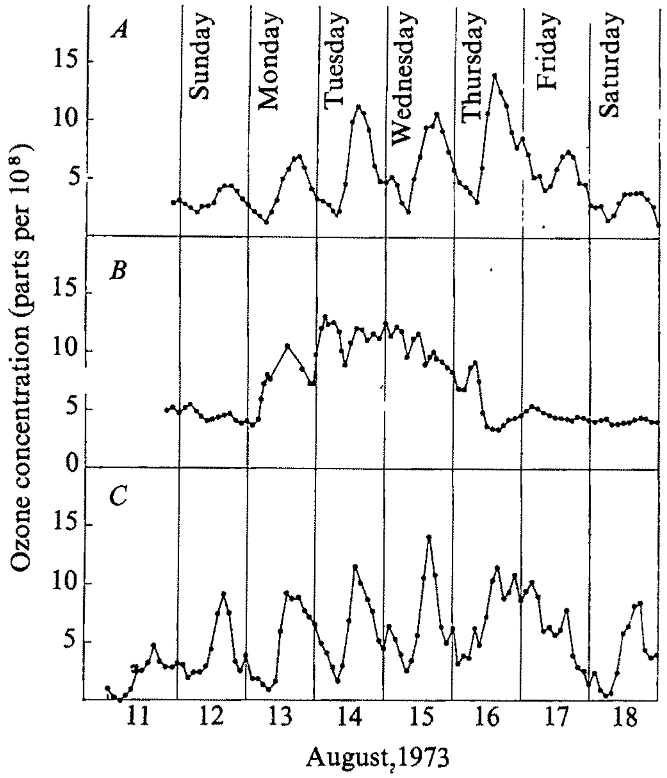


Fig. 2 Ground-level ozone concentrations during the period August 11–18, 1973. *A*, Sibton; *B*, Adrigole; *C*, Harwell.

400 m from the sea. At coastal locations, nocturnal inversions are less common. Moreover, ozone is destroyed more readily at land surfaces than at sea surfaces[6–8], thus, the difference in the patterns of diurnal variation between Adrigole and other sites can be explained. The diurnal pattern of photochemical ozone concentrations in urban areas has been attributed to the diurnal variation of insolation, with each day treated as an episode. Measurements at stations very near the coast may demonstrate the continuity of the photochemical process over several days.

Transport of ozone

During the period August 11–August 18, 1973 an anticyclone became established over the North Sea, replacing a south-westerly air flow from the Atlantic by a predominantly easterly flow of continental air over the southern British Isles. The stable weather, with nearly cloudless days was interrupted by the eastward passage of a weak cold front over the entire region during August 16 and 17, after which the air flow was predominantly westerly. The changes in air flow during the period are illustrated in the back-track air mass trajectories for Adrigole and Sibton (Fig. 4).

The ozone concentration data for August 11–18 (Fig. 2) show the presence of elevated ozone concentrations at all three sites by the time the arriving air masses had been in transit over the continent for at least 24 h. After the passage of the cold front and the advection of air directly from the Atlantic, the ozone concentrations declined again to near normal values.

It is clear that the elevated concentration of ozone observed during the episode at Sibton, situated 10 km from the Suffolk coast in sparsely populated country, must have had its origin in continental sources outside the UK. The trajectory and CCl_3F data also confirm that advection from the continent led to the high ozone concentration at Adrigole. Contribution from the UK was also negligible; the air mass trajectories, although less reliable than those at Sibton because of the paucity of observations nearby, back-track to the south-east, towards France. The sources of emissions giving rise to the high values at Harwell are less clear. The maximum levels observed at Harwell are, however, not significantly different from those at Sibton, Adrigole or even central London (0.136 p.p.m. on August 15, 1973) showing that by August 15 a sufficiently high concentration of pollutants had accumulated in air of continental origin to give similar elevated concentrations over much of southern Britain and Ireland, and probably over more extensive areas also. It is not possible, therefore, to distinguish the specific contribution to the total pollution of sources in the UK.

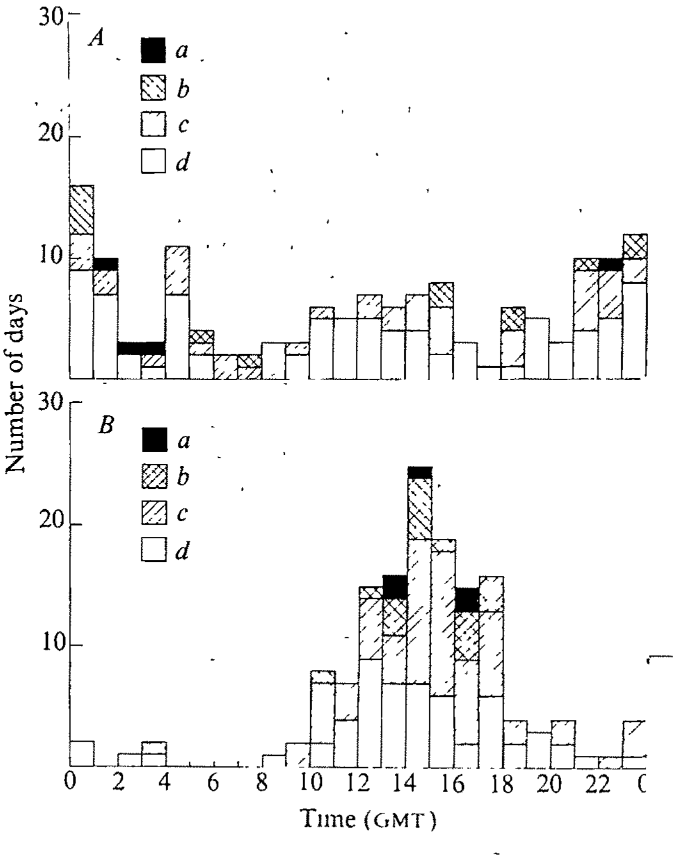
The data obtained at Adrigole show that elevated ozone concentration can be sustained in air masses several hundred kilometres from the sources of emissions, which in turn indicates a relatively long natural lifetime for ozone in the lower troposphere. The overnight persistence of the elevated ozone levels requires a lifetime of at least 10 h. Table 3 and Fig. 4 show that advection to Ireland required from 30–100 h.

Considering that the Adrigole values were approximately as high as those in England, this implies that the effective lifetime of ozone is of the order of 1–2 d. It is not possible from these limited data to disentangle the several factors controlling concentration (source strength, vertical dispersion, ultraviolet intensity, removal rates and so on). Table 3 shows, however, that the highest values at Adrigole on August 14, 1973 coincided

Table 3 Trajectory length (km) and transit time from European continent to Adrigole

Date	Time	O_3 (p.p.m.)	Length (km)	Time of transit (h)	Speed (km h^{-1})
13	1200	0.10	700	48	14.6
14	1200	0.12	700	33	21.2
15	1200	0.11	655	48	13.7
16	1200	0.05	1,310	96	13.7

Fig. 3 Time of maximum hourly mean ozone concentrations during the period May 4–October 2, 1973. *A*, Adrigole; *B*, Harwell. In both cases: *a*, maximum concentration greater than 0.11 p.p.m.; *b*, 0.08–0.109 p.p.m.; *c*, 0.05–0.079 p.p.m.; *d*, 0.00–0.049 p.p.m.



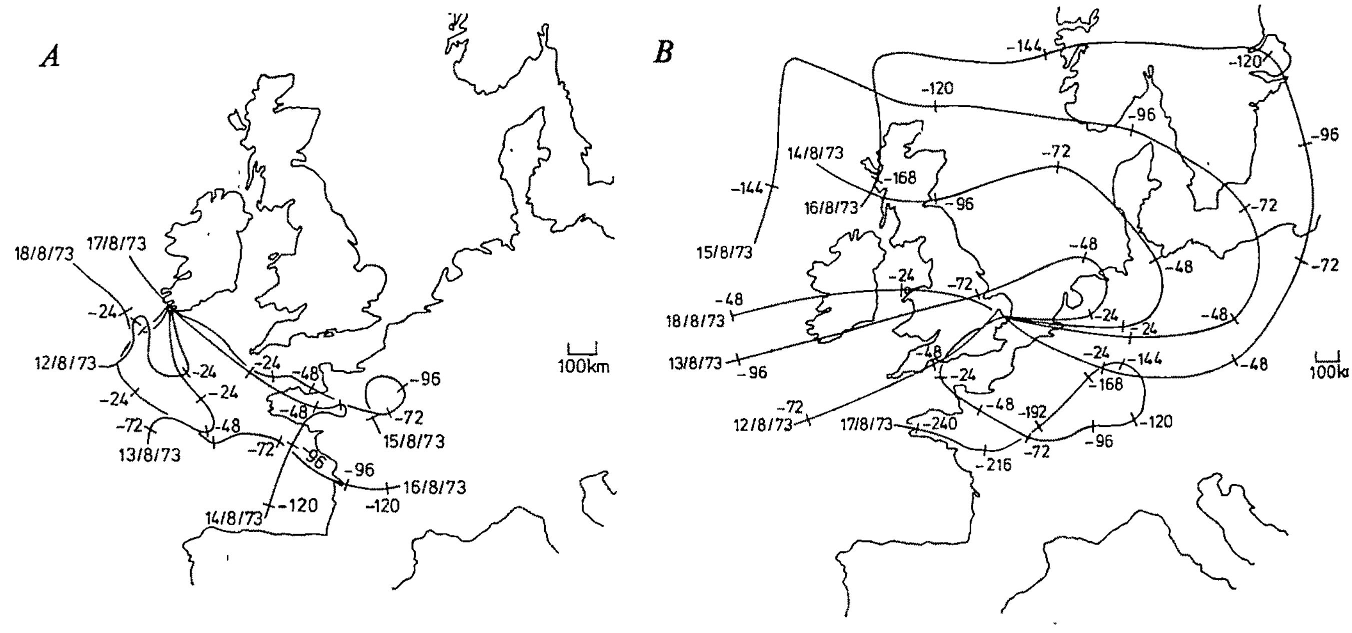


Fig. 4 Back-track air trajectories for 1200 GMT each day for Adrigole (*A*) and Sibton (*B*), together with position of air mass on preceding days.

with the shortest transit time over the sea; on August 16, however, the values, even before the frontal passage, had lowered by about 0.05 p.p.m. and were associated with trajectories and transit times twice those observed on August 14 and 15, 1973.

The alternative process of long range transport of pollutant precursors, with ozone formation occurring in response to local meteorology in each locality, is inconsistent with the high nocturnal ozone concentrations found at Adrigole. It would also require the formation of very similar ozone concentrations each day from what must be different precursor concentrations in Suffolk, central London, Oxfordshire and south-western Ireland.

We note, without comment on its relevance to the UK or Europe, that the US Air Quality Standard for photochemical oxidants was exceeded on a number of days during 1973 at all three sampling sites, including one situated in southern Ireland. We conclude that in suitable meteorological conditions measurable amounts of photochemical pollution of anthropogenic origin are transported over distances up to at least 1,000 km in north-western Europe and that on occasions continental emissions provide a major contribution to photochemical pollution in the UK. During such periods photochemical pollution is essentially a regional rather than local problem with uniformly elevated concentrations of photochemical ozone being observed simultaneously over different parts of the UK. It follows that, should controls on the emission of the pollutant precursors (hydrocarbons and nitrogen oxides) be considered necessary in order to reduce future levels of photochemical oxidants in the UK, then such controls are unlikely to be effective in the absence of similar controls elsewhere in Europe.

We are indebted to Mr Michael O'Sullivan and to the staff of Blyth Rural District Council, who maintained the monitoring equipment at Adrigole and Sibton, respectively. We thank the East Anglian Water Company for permission to use the Lodge Wood Water Tower, Sibton. Air mass trajectories were prepared by Mr J. Heffter of the Air Resources Laboratory. One of us (J. E. L.) thanks Shell Research Ltd. and the Manufacturing Chemists Association for financial support for part of this work. The ozone measurements at Sibton, Harwell and Adrigole were carried out as part of a programme of atmospheric pollution research sponsored by the UK Department of the Environment.

Received January 20; accepted March 27, 1975.

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Construction of bacterial flagella

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The flagella of Salmonella *can adopt a number of distinct helical forms. This article discusses the design of a subunit which packs to give a helical filament. Polymorphism is explained by small changes in the geometry of the subunit.*

BACTERIAL flagella are constructed from molecules of the protein flagellin by a process of self-assembly[1-6]. It is easy to see how a straight filament can be polymerised in this way, with the subunits arranged in a regular array in identical environments, as for example in tobacco mosaic virus[7]; but bacterial flagella in general have a helical shape, and it is hard to see how this can

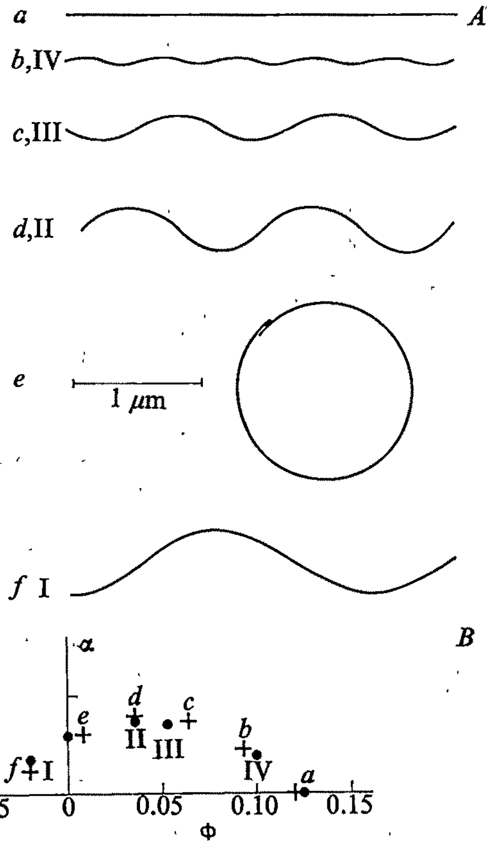

Fig. 1 *A*, Distinct helical forms made by copolymerisation of monomers *SJ 670* and *SJ814* (after Asakura[8,9]). Roman numerals are Asakura's identification, but lower case letters (*a–f*) are used for the corresponding theoretical classes proposed in this paper. Form *e* is circular, or closecoiled. Monomer *SJ670* can build *e* or *f*, and *c* and *d* if the *p*H is changed; and monomer *SJ814* can build *a* or *b*. Copolymerisation produces forms *b* ,*c* and *d*. The thickness of the filaments is roughly to scale. *B*, Curvature–twist plot for the six filaments shown in *A*. ●, Mean values from Asakura's[8,9] data (but twist of straight filament from O'Brien and Bennett[11]). +, Proposed model.

be achieved by bonding identical units to each other in a regular manner[3]. Furthermore, flagella of various strains of *Salmonella typhimurium* adopt, in different environments, a number of distinct polymorphic forms with different helical parameters[8,9].

I attempt to resolve this problem by designing a building block, within the framework of classical mechanics, which will assemble in quantity into a helical filament. This unit connects to six neighbours: four at an outer level, near the surface of the filament, and two at an inner level. When connected only by the outer contacts, the units can assemble into a family of distinct helical waveforms which correspond closely to those observed. The function of the inner connections between units is to select a particular member of the family. The model predicts a simple relationship between the total number of polymorphs and the number of longitudinal rows of subunits in the helical surface lattice.

Selected summary of present knowledge

The surface lattice of a bacterial flagellum is made up of a number of helical families, of which the most important are one-, five-, six- and eleven-start helices (Fig. 2*a*). This lattice is common to filaments from all strains so far investigated[10-12]. The main facts about the waveforms of the filaments have been reviewed by Asakura[8] and Asakura and Iino[9]. Figure 1*A* shows the distinct waveforms observed when flagella are constructed *in vitro* from monomer obtained from strains *SJ670* and *SJ814*, both separately and mixed[8,9].

From measurements on the waveform of a given filament it is possible to deduce, by simple geometry, the uniform twist and curvature which must be imported to the filament by the local building process. Here twist is described by the inclination φ (radians) of the longitudinal helices in the surface lattice to the local axis of the filament, and curvature α by the (percentage) difference in length between the longest and shortest of these longitudinal columns; I take the diameter of the filament, nominally, as 20 nm. It is useful to describe the results in the form of a plot of curvature against twist; Fig. 1*B* shows the data for the waveforms of Fig. 1*A*. The diagram incorporates recent observations[13] on the helical handedness of various waveforms. The circular filament has zero twist. In the case of a

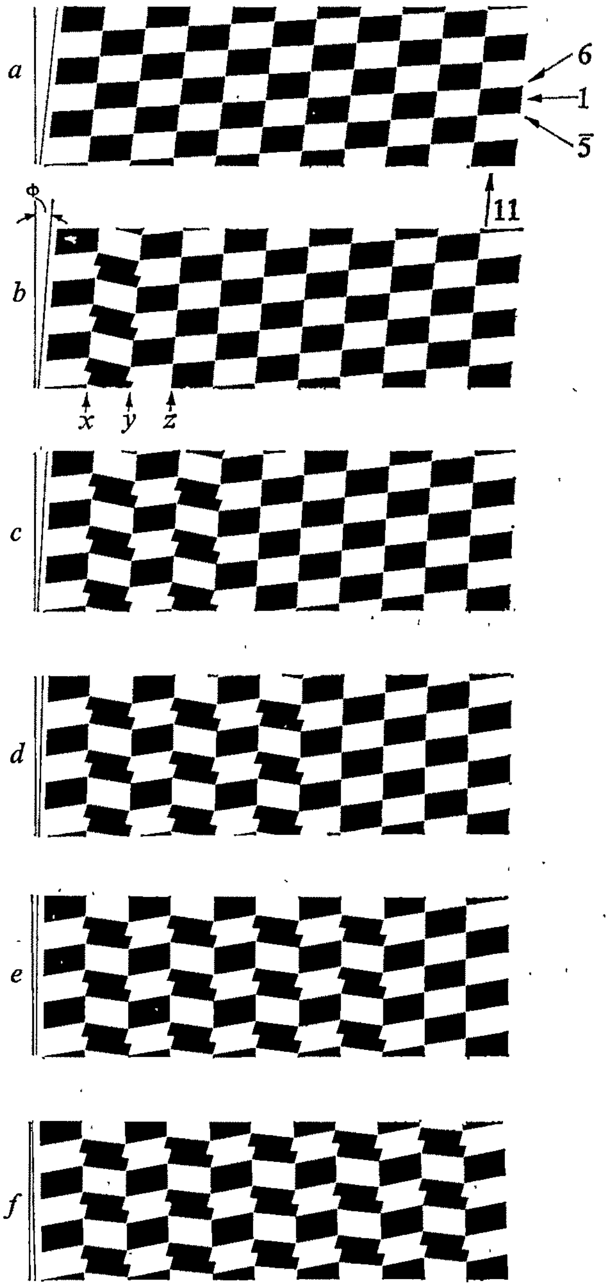

Fig. 2 Surface packings corresponding to the six ideal waveforms (*a–f*) viewed from the outside. The lower and upper edges of the patches each form a circle when the packings are wrapped into a cylindrical surface. A member of the one-, five-, six- and eleven-start helices in the surface lattice is picked out in *a*. Longitudinal (eleven-start) lattice lines make angle φ with the local axis; these two directions are shown at the left. Curvature is produced by small differences in axial length between 'strands', that is, lines of contacts, *x*, *y*, *z*, marked in *b*. Inner connections between units are not shown.

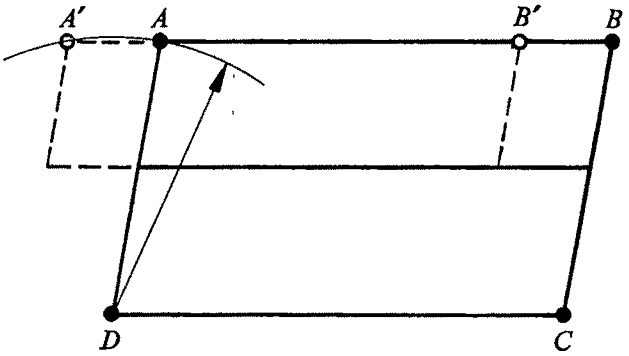

Fig. 3 Layout of connecting points on flat units in Fig. 4. ●, Normal connections; ○, alternative connections. The unit has two stable configurations involving relative motion of two parts of the unit, which are connected by interior bonds (not shown) having a bi-stable feature. The units fit exactly in the plane arrays of Fig. 2, but the design could easily be altered so that $A'D$ and $B'C$ are slightly less than AD and BC respectively.

straight filament the curvature is zero, and the twist can only be found from a knowledge of the surface lattice; O'Brien and Bennett[11] have found the twist of straight filaments of *SJ814* by high resolution electron microscopy using optical filtering techniques[14].

Surface packings and unit design

Consider the surface packings of identical flat units shown in Fig. 2. In *a* the interunit connections are at the corners of the quadrangular units, giving a uniform packing, but in *b–f* units in some columns have undergone a change in configuration which makes them rotate forwards. This change is here represented as a shear in the tetragonal disposition of the bonding sites (Fig. 3). The effect of rotation of units in a column is to shear the lattice along a nearly axial line of units, thereby altering the tilt of the columns with respect to the base-line, and thus changing the twist of the assembly. Each column so rotated gives an additive contribution to the twist of the assembly. A special feature is that rotated columns are clustered but are not adjacent to each other; I shall explain this later.

Now the two configurations of the unit shown in Fig. 3 are arranged so that the longitudinal dimensions are the same, that is, $A'D = AD$ and $B'C = BC$, and the units in the plane arrays of Fig. 2 fit exactly. It would be easy, however, to arrange for these dimensions to be slightly different, so that a longitudinal strand of connections such as x or y (Fig. 2*b*) is slightly shorter (say) than a strand z of regular bonding. If in addition the units are given a degree of longitudinal elasticity the units will assemble, in the round, to a curved filament. Indeed, with units having these features it is easy to show by the methods used for analysing thermal stress in elastic structures[15] (the difference in unstressed lengths of the strands being analogous to thermal expansion) that the curvature of filaments built according to the six patterns *a–f* is proportional to sin $2n\pi/11$, where n is the number of rotated columns, whereas the twist (relative to the twist for pattern *a*) is proportional to n. Hence a plot of curvature against twist gives a sinusoidal, discrete variation (Fig. 1*B*).

If we assign a reasonable degree of tolerance for the distortion of the filaments in the course of preparation for microscopy[8,9], we can see that the surface states *a–f* in Fig. 2 correspond closely to the waveforms observed by Asakura. This correspondence forms the basis of my proposed model.

Features of the surface packing

There are several crucial features of the packings shown in Fig. 2, without which the model would not work. First is the ability of the units to adopt two stable attitudes. The unit of Figs 2 and 3 has an internal movement controlled by a bi-stable 'switch' analogous to that already described[16] at the α–β interface in a haemoglobin molecule.

Second, each unit is connected to only four neighbours. A coordination number greater than this would inhibit the mechanism involved in changing between states in the surface arrays. This follows from the kinematics of interconnected rigid bodies: a full description will be given elsewhere.

Third, since the packings involve a chequerboard pattern, the longitudinal length of the assembly is determined by the strands of connections between adjacent columns of units (for example, x, y, z in Fig. 2*b*) rather than the individual columns themselves. This accounts (see next section) for some special features.

The notions that there are two inherently stable conformations of the subunit, and that different helical waveforms correspond to different numbers of longitudinal columns in the two states have been put forward by Asakura[8], and elaborated by Wakabayashi and Mitsu[17] who indeed did an elastic calculation on the curvature resulting from different numbers of columns in an alternative configuration. The absence of detailed consideration of interelement bonding from this analysis[17] (my crucial features two and three) however, prevented them from taking their model beyond a 'phenomenological' stage.

Why do 'rotated' columns cluster?

I have explained that the difference in the dimensions AD and BC between the two alternative configurations of the units gives a shortening of strands on one side of the filament and consequently a curvature. There is in fact a stringent geometrical requirement that the relative lengths of the strands of connections should vary sinusoidally around the circumference (Kirchoff's hypothesis[18]). Consequently each strand must be distorted a little from its natural long or short configuration to make it fit (except in case *a*). There will thus be an elastic strain energy of distortion[15] associated with any given packing, and it is easy to show quantitatively that, for a given number of columns in the alternative configuration, the strain energy is least when they are clustered precisely as shown in Fig. 2*b–f*. Figure 4 shows an example of this. The minimum is deep, and it is reasonable to suppose that this is why other packings are excluded.

Inner connections between the units

The outer connections described so far enable the units to assemble in regular packings like those of Fig. 2*a* but having an

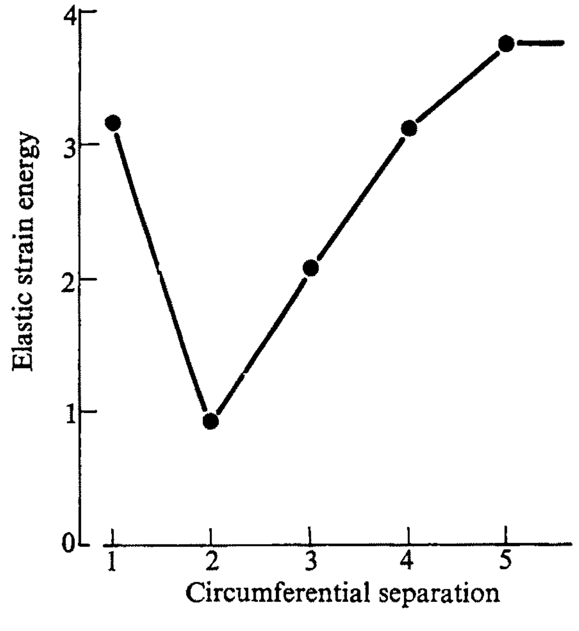

Fig. 4 Strain energy of distortion of a packing involving two columns of rotated units, disposed in various ways. Circumferential separation is counted in numbers of columns; a value of 1 means that the columns are adjacent, and so on. Arbitrary but consistent units of elastic strain energy.

arbitrary number of longitudinal columns around the circumference. We can make the packing contain just 11 columns by insisting that adjacent units make an angle of $360°/11 \simeq 33°$ when viewed along the local axis. This can be done in various ways, all involving inward-facing projections on the units, with interunit connections at the tips. In fact the angular rotation of consecutive units on one of the six-start lattice helices (say) is slightly different for each of the six packings shown in Fig. 2, varying from 34.7° in *a* to 32.4° in *f*. Thus a fine control of this angle, by small changes in conformation of the inward projections, can determine which of the packings is to be built: a wrong packing would not allow simultaneous bonding between the inner and outer structure of the assembly[3].

Design difference in flagellin molecules

I suggest that the flagellin molecules from different strains of *Salmonella* all have nearly the same shape. The observed forms of the filaments shown in Fig. 1 and elsewhere[8,9,12,19,20], can all be understood in terms of small changes in configuration of the unit. These might occur in a given strain in response to a change in the *p*H (compare ref. 7) or as differences between the flagellin molecules of different strains resulting from changes in a few residues on the polypeptide chain.

I have described the essential features of the model; but various other features are not essential. For example, the four outer bonding sites need not form a plane figure; an arbitrary tetragon has essentially the same packing properties. Also, the alternative configurations of the unit need not involve a change within the unit; it is also possible to make the model work by having a bi-stable bonding site at one of the corners of the unit. Anyhow, the design features of the subunit are consistent with what is known about the chemistry and morphology of protein molecules. More details of the three-dimensional geometry of packing, and a closer examination of experimental data[9,12,19,20] will be presented elsewhere.

Dr A. Klug suggested this problem to me, and I thank him for sustained help. I thank Dr S. Asakura and Dr T. Iino for sending advance reports, and Messrs A. A. Barker, E. J. W. Taylor and R. Denston for help in making physical models.

Received February 4; accepted March 26, 1975.

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letters to nature

Significance of the angular diameter-redshift relation

Hewish, Readhead and Duffett-Smith[1] have reported that interplanetary scintillation techniques reveal an absence of small-diameter sources at the largest redshifts. They have discussed the cosmological implications of the result using standard, homogeneous Friedmann models of the Universe. Here I point out the important effects on the angular diameter–redshift relation caused by small scale inhomogeneities.

The observational relations in the standard, homogeneous Friedmann models (HFM) have been generalised by Dyer and Roeder[2] to take into account the fact that the Universe may be quite inhomogeneous on the scale of galaxies and clusters of galaxies. The inhomogeneities are put into the models following the method of Schücking[3] and Kantowski[4]. In these inhomogeneous Friedmann models (IFM) the total density may be written

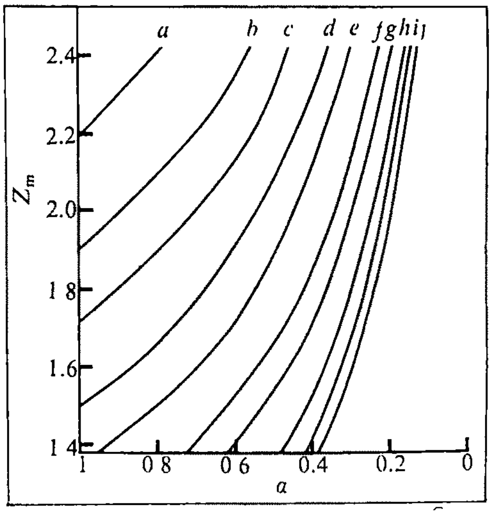
$$\rho = \rho_I + \alpha\rho \qquad (1)$$

where ρ_I denotes the density of inhomogeneities, and α, the homogeneity factor, lies in the range $0 \leq \alpha \leq 1$. If $\alpha = 0$, the model is completely inhomogeneous; if $\alpha = 1$, $\rho_I = 0$ and the model is an HFM. If the lines of sight to distant sources do not pass too close to intervening galaxies, so that shearing of the light beam can be neglected, the distance–redshift relations for the IFM can be expressed as a simple hypergeometric differential equation which gives the solid angle distance, D_s, as a function of the acceleration factor, q_0, the redshift of the source, z, and α. Since the projected area of a source, A, and the solid angle it subtends Ω, are related by

$$A = D_s^2\Omega \qquad (2)$$

it is clear that minimum angular diameter occurs at the redshift for which D_s is a maximum.

Fig. 1 The redshift of minimum angular diameter, Z_m, plotted as a function of α The various Friedmann models specified by letters in the diagram have the following values of q_0:*a*, 0.1, *b*, 0.15; *c*, 0.2; *d*, 0.3; *e*, 0.4; *f*, 0.6; *g*, 0.8; *h*, 1.2; *i*, 1.6; *j*, 2.0.



The redshifts of minimum angular diameter, Z_m, have been calculated as a function of q_0 and α in the zero-shear IFM (Fig. 1). The determination of Z_m is, in itself, not sufficient to determine q_0. Thus, for example, if $Z_m = 1.6$, then $0.25 \leq q_0 \leq 2.0$ corresponds to $1 \geq \alpha \geq 0.3$; and to determine q_0 requires that α be specified. Note that if $\alpha = 0$ there will be no minimum angular diameter regardless of the value of q_0.

The interpretation of further observations of the angular diameter-redshift relation may provide a simple test for the existence of an intergalactic medium, if the acceleration factor can be estimated independently.

This work has been supported by the National Research Council of Canada.

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Received February 13; accepted April 1, 1975.

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Harmonic analysis of the light of DQ Herculis

SEVERAL classes of interacting binary stars, such as the dwarf novae, nova-like variable stars and X-ray stars, present many examples of rapid periodic optical variations. DQ Herculis (nova 1934) is, however, the only nova known to show such variations. They are of low amplitude, generally less than 0.04 mag semi-amplitude, and have a period of 71.065459 s (refs 1–3). We report here a study of the harmonic components of the 71-s periodicity, and show that the results place severe limits on the available models of the variation.

The observations were made in June, August and September, 1973 at the Cassegrain focus of the 2.08-m telescope of the McDonald Observatory using the high speed two-channel photometer developed by Nather and Warner[4]. The data consist of six observing runs totalling 14 4 h of photometry of DQ Herculis; data taken during eclipse were not included. Individual power spectra were computed for each of the observations and these spectra were then averaged together to produce the power spectrum shown in Fig. 1. The frequencies corresponding to the 142-s subharmonic and to the higher harmonics of the 71-s variation are indicated.

The prominent peak corresponds to the 71-s periodicity and is nearly three orders of magnitude above the noise. At the subharmonic frequency, the contribution to the power spectrum is consistent with the surrounding noise level, which is dominated by the optical flickering of DQ Her. The power at the subharmonic frequency is less than the power at the fundamental frequency by a factor of 310 (17.6 in amplitude).

There are no highly significant harmonic components to the 71-s variation. There is, however, evidence for the existence of the first harmonic, since the highest point of the 1,680 points in the power spectrum that lie at frequencies higher than 0.016 Hz is found precisely at the frequency of the first harmonic. After subtraction of noise, it is found that the contribution of this component is less than that of the fundamental by a factor of 600 in power (24 in amplitude). These values correspond to a maximum semi-amplitude for the first harmonic of 0.0017 mag. The remainder of the observed power spectrum has a shape and level consistent with white noise. A conservative upper limit to periodic components in the frequency range of 0.028–0.1 Hz is 0.0014 mag.

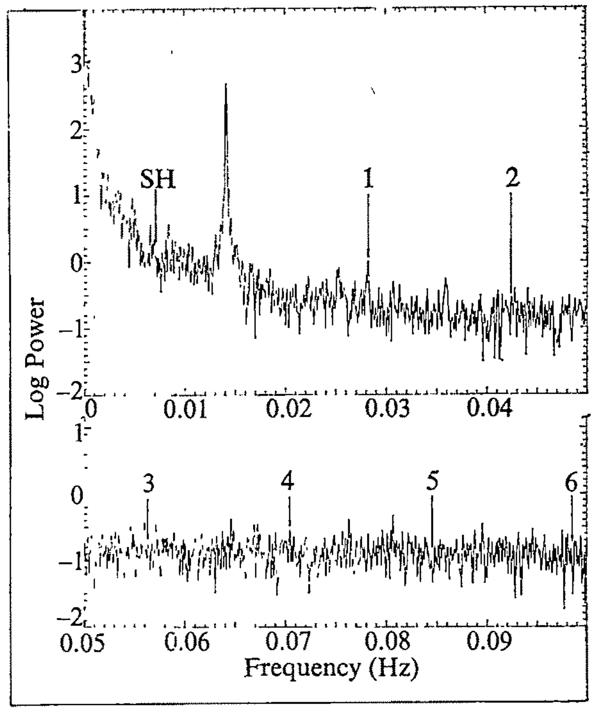

Fig. 1 Log of averaged power of the light of DQ Her showing the harmonic character of the 71-s periodicity. Frequencies corresponding to the 142-s subharmonic (SH) and to the higher harmonics (numbered) are indicated by arrows.

Two major groups of models have been advanced to explain the 71-s variation of DQ Her: non-radial white dwarf pulsations and a rotating white dwarf with a dipole magnetic field[3,5–8]. In general, a rotating white dwarf with a dipole magnetic field should produce a 142-s subharmonic because of physical and geometric asymmetries between the two poles. On the other hand, pulsational models could also require significant contributions to the power at the harmonic frequencies. Our study has shown that the 71-s variation of DQ Her is a sinusoid of remarkable purity, with a small harmonic contribution to the power occurring only at the frequency of the first harmonic. Consequently, no model of the variation can be considered complete unless it is consistent with this purity.

We thank Mr John McGraw for assistance and discussion, and Dr E. L. Robinson for comments and suggestions.

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Received February 24; accepted April 1, 1975.

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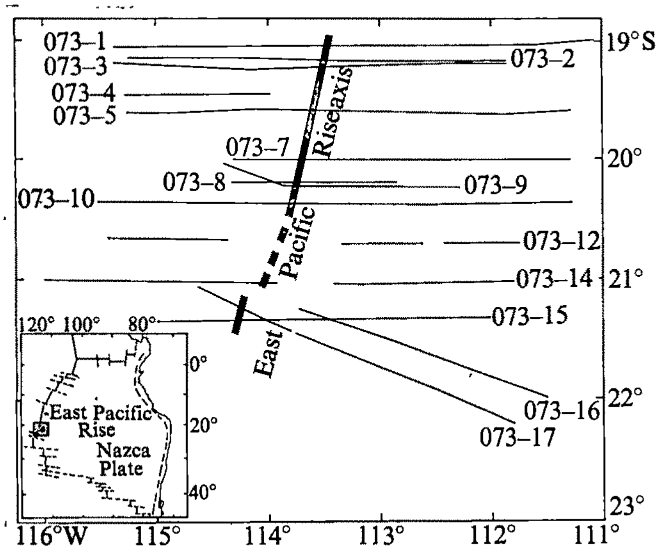
Short-wavelength magnetic anomalies in a region of rapid seafloor spreading

THE main features of the geomagnetic time scale for 0–3 Myr BP are well defined[1–3], but the existence and/or number of short events (polarity intervals lasting less than 50,000 yr) is still under discussion. Interest in correlating these short events with features in marine magnetic profiles has focused on three short-wavelength anomalies: anomaly X[4,5] which appears at approximately 2.3 Myr BP; anomaly W[5–7] at 2.0 Myr BP; and a short-wavelength feature in the centre of the central anomaly[3,6,8,9]. These anomalies appear rarely, and then usually only in profiles from regions of rapid spreading. We present here an analysis of a remarkable new set of data from just such a region. Our results provide evidence of the existence of two, or possibly three, short events in the interval 0–3 Myr BP, and of the existence of a magnetised ridge at the precise centre of seafloor spreading.

Our data consist of 14 profiles from a survey of the East Pacific Rise (EPR) crest, centred at 20°S (Fig. 1), where the total spreading rate is about $161\ \mathrm{mm\ yr^{-1}}$. This survey was completed in the spring of 1973 by the NOAA ship Oceanographer. Navigation was entirely by satellite. Total-field intensity data collected during this survey were reduced to anomaly form[10], and data collected in the vicinity of seamounts or of obvious structural disturbances in the seafloor were discarded. The remaining profiles were projected on to a great circle parallel to the spreading direction—103°—and redigitised to a constant sample interval. These profiles were then transformed to the pole using the method of Blakely and Cox[7], and adjusted to a uniform spreading rate between known[3] magnetic events: the Brunhes–Matuyama reversal boundary at 0.7 Myr BP, the Jaramillo Event centred at 0.92 Myr BP, the Olduvai Event centred at 1.73 Myr BP, and the Matuyama–Gauss reversal boundary at 2.41 Myr BP. Stacking the resulting profiles reduces the random noise in the data set and enhances coherent magnetic anomalies[11,12]. Signals other than those from magnetic reversals, such as from linear topographic features parallel to the rise axis or from intensity fluctuations of the palaeofield[13], would also be enhanced.

Fig. 1 Trackline map of the 20°S area over the East Pacific Rise. Inset, location of survey area.



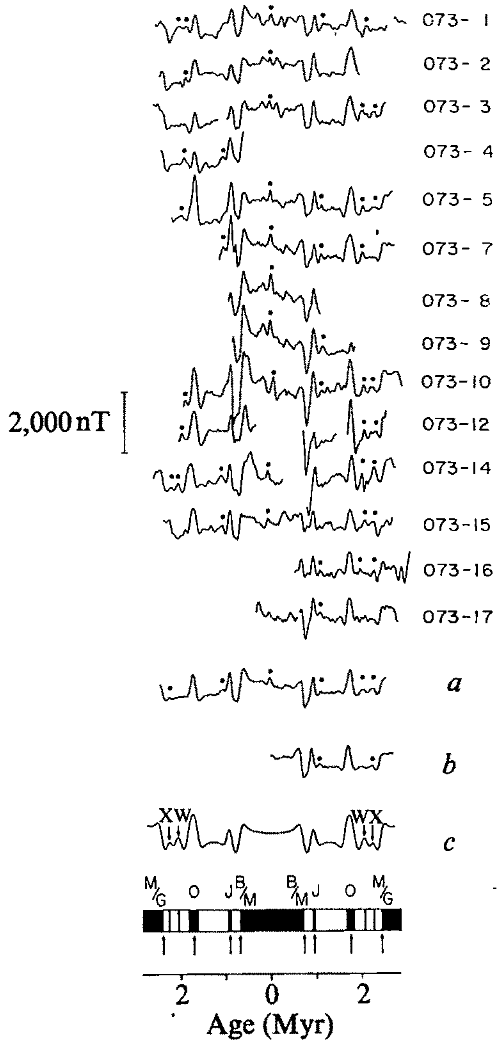


Fig. 2 Individual magnetic anomaly profiles from tracklines shown on Fig. 1. The profiles have been transformed to the pole and adjusted to a uniform spreading rate. *a*, Average of all 14 profiles; *b*, average of profiles after folding in middle; dots, short-wavelength anomalies of controversial origin discussed in text. *c*, Model; arrows, points of 'known' age[3]; black, normal polarity. B/M and M/G Brunhes–Matuyama and Matuyama–Gauss reversal boundaries, respectively; J and O, Jaramillo and Olduvai events, respectively. The model profile (*c*) was calculated from a crustal layer with top and bottom at 3.0 and 3.5 km respectively. Two brief normal polarity events are centred at 2.05 (W) and 2.25 (X) Myr BP.

At 20°S on the EPR, the contribution to the magnetic profile from the abyssal-hill topography is minimal except in the one case discussed here. We have no way, however, of differentiating between anomalies produced by significant palaeointensity fluctuations and anomalies produced by true, short events. For the sake of simplicity we will assume that the short-wavelength anomalies discussed here are the result of brief reversals.

Figure 2 shows the individual profiles and the resulting averages. The computer-generated profile (Fig. 2*c*) was obtained by assuming a horizontal crustal layer with its top and bottom at 3.0 and 3.5 km, respectively. To approximate the imperfection of the crustal recording of geomagnetic reversals, the magnetisation of the layer was convolved with a normal distribution[14,15] thus producing a smooth transition in the polarity of the magnetisation at any reversal boundary. A standard deviation of 4 km for the normal distribution was determined by repeated trials. The time scale used to generate the model is shown at the bottom of Fig. 2. This scale is essentially from Klitgord[3] except that two additional short events are included: the first lasting from 2.03 to 2.06 Myr BP and the second from 2.24 to 2.26 Myr BP.

The anomaly occurring on 83% of our profiles at about 2 Myr BP (Fig. 2) has been reported in other studies of marine magnetic profiles[5-7] and was labelled 'W' by Emilia and Heinrichs[5]. This anomaly probably correlates with the Reunion events, but a controversy exists about the number, age, and duration of these events. A study of volcanic rocks on Reunion Island revealed only one normal polarity event[16], although other evidence suggests that there may be two[1,17] or even three[18] normal polarity events clustered near 2 Myr BP. Some of this other evidence is, however, based in part on an assumed correlation between an apparently bimodal distribution of age polarity data within the span of about 1.95–2.15 Myr BP and the two small marine magnetic anomalies W and X[1,17].

McDougall and Watkins[16] point out that all these dates, because of their nearly overlapping errors, may correspond to the one normal event that they found at Reunion Island and which they dated at 2.02±0.02 Myr BP. They also suggest that this event corresponds to anomaly W, but that anomaly X, dated at 2.3±0.1 Myr BP[5], is significantly older than the event found at Reunion Island[16]. We concur with this suggestion. Unfortunately, because of the large transition width at the reversal boundary and because of inherent limitations on the resolution possible with marine data[11,12], we cannot determine whether anomaly W was produced by more than one event. We can only conclude that it is adequately modelled by a single short event lasting from 2.03 to 2.06 Myr BP.

The anomaly located at about 2.3 Myr BP in Fig. 2 correlates with anomaly X reported in previous marine studies[4-7] and possibly with a short event found in sediments from south-western Ethiopia[18]. Anomaly X appears on 56% of the profiles shown in Fig. 2 and is better represented on the eastern flank of the EPR than on the western flank. We conclude that this anomaly is adequately modelled by a short event lasting from 2.24 to 2.26 Myr BP.

The existence of a brief, normal polarity event about 1.07 Myr ago has been indicated in studies of sediment cores[19,20] and volcanic rocks[21,22] but to our knowledge has not been reported with any certainty[3,6] from marine magnetic profiles A small positive anomaly that would be about 1.1 Myr old appears on 52% of our profiles but is not strikingly evident in the average profiles. Our unpublished data suggest variations in the spreading regime here during Matuyama time and these variations could cause slight offsets among the profiles. Such small-scale variations in spreading rates would preclude precise stacking of this short wavelength anomaly, as with the two anomalies already discussed. The identification of this anomaly as a short event occurring 1.1 Myr ago must, therefore, remain tentative.

The spreading axis of the EPR is commonly denoted by an axial block or peak several kilometres wide and a few hundred metres high[23] In the 20°S survey area there is a small positive magnetic anomaly of 50–100 nT associated with the axial peak found on nearly all crossings of the axis (91% of the profiles in Fig 2). This anomaly is best displayed on lines 5–9 of Fig. 2 and shows up on the average profile. This small anomaly has been recognised in various areas by other authors and two causes have been proposed for it. Emilia and Heinrichs[6] proposed that the Blake reversed polarity event, dated at 0.108–0.114 Myr BP ±10% (ref. 8) caused flanking magnetic lows resulting in a relative, central magnetic high Sclater and Klitgord[9] and Klitgord[3] found a short-wavelength magnetic anomaly associated with the axial peak of the Galapagos spreading centre and related it to an excess of magnetised material within the axial peak.

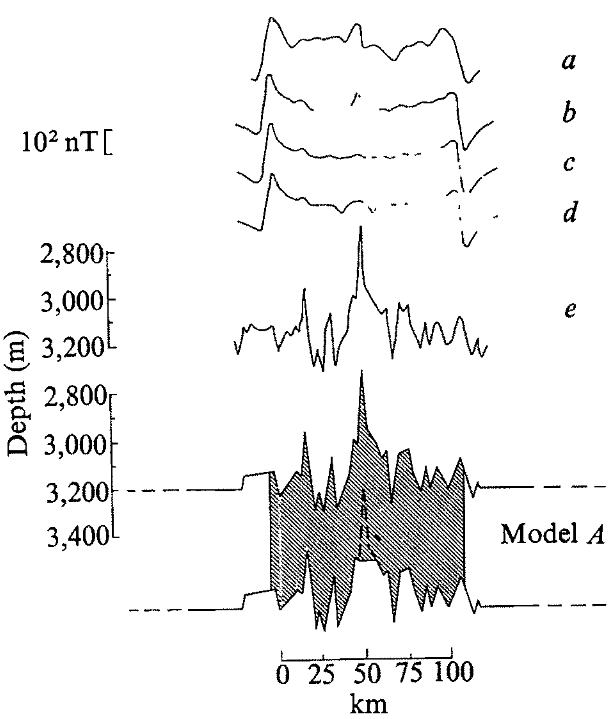


Fig. 3 Observed (*a*) and calculated (*b*–*d*) magnetic anomalies along line 073–5; *b*, assuming central peak atop a magnetised layer 3.0–3.5 km deep; *c*, assuming base of layer faithfully follows the surface; *d*, assuming structure of *c* and including Blake Event; *e*, observed bathymetry. Remanent magnetisation is 0.015 e.m.u. cm^{-3}; shaded regions, normal polarity.

In an attempt to discern which of these causes may be correct, we constructed several magnetic models of the central anomaly of survey line 5. The models all incorporate a magnetised layer 0.5 km thick that conforms to the observed bathymetry and has a uniform intensity of remanent magnetisation of 0 015 e.m.u. cm^{-3} (Fig. 3). In model *A* we have followed the suggestion of Sclater and Klitgord[9] and assumed that the central peak of the EPR rests on top of usual 0 5-km-thick basaltic basement —assumed in this case, to lie at a depth between 3.0 and 3.5 km (Fig. 3). The resulting profile (Fig. 3*b*) provides the best match of all the models which we tested, approximating the short-wavelength anomaly in both amplitude and waveform reasonably well. The second model is similar to the first, except that the central peak is considered to result entirely from block faulting so that the layer is 0.5 km thick throughout The second profile displays essentially no magnetic anomaly over the central topographic peak (Fig. 3*c*) The third model (Fig. 3*d*) is the same as the second but contains the Blake reversed polarity event; the profile calculated from this model has small negative anomalies flanking the central portion, but the overall waveform of the central anomaly is noticeably different from the observed data (Fig. 3*d*).

The close match between the calculated profile (Fig. 3*b*) and the observed profile (Fig 3*a*) suggests that the short-wavelength anomaly associated with the axial peak is the result of a pile of magnetised, extrusive basalt resting on top of a layer of uniform thickness and supports the initial presentation of this idea[9]. Furthermore, our data indicate that the central peak need not be more intensely magnetised than the adjacent material to produce the observed anomaly. As is the case with all modelling efforts, however, we could construct other more complicated models with some combination of the first and third models (Fig. 3*b* and *d*) that would also result in a calculated profile similar to those observed.

In summary, magnetic anomaly data from the EPR at 20°S, where the total crustal accretion rate is 161 mm yr^{-1} reveal several short-wavelength magnetic anomalies. The oldest of these is about 2.25 Myr old and is correlated with the previously reported anomaly X[4,5]. A second small anomaly approximately 2.05 Myr old may be the result of one or two closely spaced Reunion events and is correlated with marine magnetic anomaly W[5]. A brief period of normal polarity about 1.1 Myr ago may be reflected by some individual profiles (Fig. 2) but does not show up well in the average profile. Finally, a short-wavelength anomaly always found in conjunction with the topographic axis of the EPR apparently results from an excess of uniformly magnetised material at the rise axis.

We thank B. H. Erickson who was responsible for the collection and initial processing of the data and Allan Cox for reviewing the manuscript. This work was supported by the US National Science Foundation through the IDOE Nazca Plate Project.

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Received February 10; accepted March 2, 1975.

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Plate tectonic and palaeomagnetic implications for the age of the Deccan Traps and the magnetic anomaly time scale

IF reconstructions of plate tectonic movement based on magnetic anomaly data are found to be inconsistent with reconstructions based on palaeomagnetic data (see refs 1–3) it may indicate that one or more of the basic assumptions of the plate tectonic model is erroneous; the age or identification of a particular anomaly may be incorrect, the pole positions or sample ages may be unreliable, or there may be an error in assessing plate rigidity. We present an example of inconsistency between the two types of data and explore its implications for the chronology of magnetic reversals during the late Cretaceous and early Tertiary and for the age of the Deccan Traps.

We have previously[4] reconstructed the major plates in the Atlantic and north-eastern Indian Ocean at the time of anomaly 24 (approximately 60 Myr ago according to the magnetic anomaly time scale of Heirtzler et al.[5]). Anomaly 24 is particularly well defined in these oceans, and we estimated that the uncertainty of the relative positions of the plates is about 200 km. Yet when palaeomagnetic poles for Europe and India, implicitly assumed also to be 60 Myr old, are reconstructed according to the positions of anomaly 24 in the Atlantic and Indian Oceans, the pole positions fall short of one another by more than 2,000 km (Fig. 1). It seems reasonable to assume that the plates are rigid, as the only obvious internal deformation has occurred in the East African Rift System and is clearly negligible compared with the discrepancy obtained. Possibly the best sets of palaeomagnetic data are from the North Atlantic Tertiary Volcanic Province and the Deccan Traps, (Table 1, Fig. 1)[6,7]. Thus, the age of either the North Atlantic Tertiary Volcanic Province or the Deccan Traps, or anomaly 24, must be significantly different from 60 Myr.

The dating of the North Atlantic Tertiary Volcanic Province has been particularly thorough. Nearly all of the measured potassium–argon and rubidium–strontium determinations have given ages of 50–65 Myr (Table 1).

The age of the Deccan Traps has been less well determined. Although measured ages range from 40–65 Myr (refs 8–10), argon loss seems to have occurred in some of the samples, and the Deccan Traps could have been extruded entirely between 60 and 65 Myr ago[6,8,10]. Thus, although the few radiometric ages that exist for the Deccan Traps may indicate that both the Traps and the North Atlantic Tertiary Igneous Province were erupted simultaneously the Deccan Traps may, in fact, be a few million years older. At the same time, recent revisions of the magnetic time scale assign to anomaly 24 an age of 56 Myr (ref. 11) or 53 Myr (R. L. Larson, personal communication). Thus, uncertainties in the ages of both the Deccan Traps and anomaly 24 allow some flexibility in reconciling differences of calculated pole positions at the time of anomaly 24. In fact, because of the especially rapid motion of India with respect to both the pole[12] and the surrounding plates[13] during the late Cretaceous and early Tertiary, the ages of both the Deccan Traps and anomaly 24 are critical; an older age for the Deccan Traps or a younger

Fig. 1 Equal area projection of present palaeomagnetic pole positions from the North Atlantic Province (crosses); present Deccan Trap pole positions (open circles); reconstructed pole position of Deccan Traps (in their positions relative to Europe at the time of anomalies 24 and 32, respectively), (closed and dotted circles).

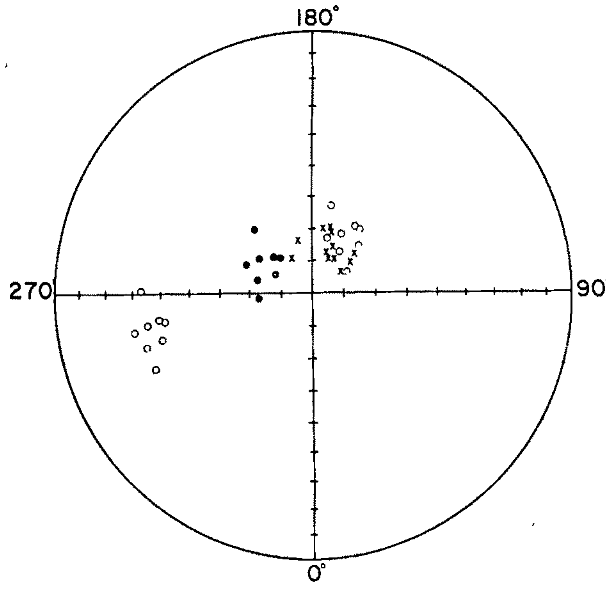

Table 1 Selected palaeomagnetic poles and ages from the North Atlantic Tertiary Volcanic Province and the Deccan Traps*

Rock unit	Age (Myr)	Ref.	Pole Latitude (°N)	 Longitude (°E)	Ref.
North Atlantic Tertiary Volcanic Province					
Faeros Island volcanics	53–59	14	77	161	15
Skye lavas and intrusives	54±3	16	74	157	17
Ardnamurchan gabbro	55±6	18	69	165	17
	60±3	19, 20			
Rhum gabbro			69	171	17
Mull intrusives	60±10	18	72	133	21, 22
Arran dykes	55–65	23	78	149	24
Antrim igneous suite	49.9±2.2	25	78	145	26
Lundy dykes	52±2	27, 28	75	130	29
Ayrshire dykes			79	128	30
Tertiary dykes, Scotland	34–57	31	73	197	31
Tertiary dykes, UK			77	211	32
St Kilda	56–64	33			
Northern Ireland igneous rocks			70	163	34
Deccan Traps					
Deccan Traps (McElhinny's average)			30	281	6,12
Amarkantak lavas			33	294	35
Mount Pavargash lavas	59–63	9	39	286	36
	66	10			
Aurangabad lavas			33	287	37
Deccan Traps, western Ghats			35	280	38
Mount Gima lavas and intrusives	65	10	41	280	39
Mahabeleshwar lavas	≥61.5	10			
Malwa Plateau lavas			33	269	40
Jalna lavas			39	279	41

* McElhinny[4] and Bott[42] were used as guides for selecting and combining these data.

age of anomaly 24 will reduce the discrepancy in reconstructed pole positions.

To explore this further we reconstructed the positions of the lithospheric plates with respect to Europe at the time of anomaly 32, with assigned ages of about 75 Myr (ref. 5) and 70.5 Myr (ref. 11). The average pole positions again disagreed, by about 600 km, but in the opposite sense from the disagreement obtained using anomaly 24. The age of the Deccan Traps is apparently less than that of anomaly 32.

Because Europe moved relatively little with respect to the pole during the Tertiary and Cretaceous[6], the North Atlantic Tertiary Province probably moved very little with respect to the pole during the period when anomalies 24 to 32 formed Assuming this, we can use a reconstruction that brings the two sets of palaeomagnetic poles into approximate coincidence to constrain the age of the Deccan Traps or the anomalies. Assuming an approximately constant rate of motion between India and Europe between the times of anomalies 24 and 32, the age of the Deccan Traps can be estimated for various proposed ages of anomalies 24 and 32. Similarly, using given ages of the Deccan Traps and one of these anomalies, the age of the other anomaly can be estimated. Table 2 summarises several combinations of assumed and calculated ages.

Using the time scale of Sclater *et al.*[11], the Deccan Traps are calculated to be only 68 Myr old (line *b*, Table 2). If we accept the age found by Sclater *et al.*[11] for anomaly 32 but Larson's age determination for anomaly 24 (personal communication), then the calculated age for the Deccan Traps is 67 Myr (line *c*, Table 2). Given the uncertainties in all of the ages, reconstructions, and palaeomagnetic poles, 67 or 68 Myr is probably indistinguishable from the measured ages of 60–65 Myr[8–10].

Similarly, assuming that the Deccan Traps are 65 Myr old, then anomaly 32 becomes younger than 70 Myr old (lines *d* and *e*, Table 2). A small change in the assumed age of the Deccan Traps produces a large effect on the age of anomaly 24 (compare lines *b* and *c* with line *g* in Table 2). Because of the uncertainties in the dating of rocks, an accurate determination of the age of anomaly 24 by this method will be difficult. Nevertheless, anomaly 24 must be younger than 60 Myr old.

Table 2 Assumed and estimated ages (Myr) of anomalies 24 and 32 and of the Deccan Traps

	Deccan Traps	Anomaly 24	Anomaly 32
a	72	60 (5)*	75 (5)
b	68	56 (11)	70.5 (11)
c	67	53†	70.5 (11)
d	65 (9)	53†	68
e	65 (9)	56 (11)	66
f	65 (9)	25	75 (5)
g	65 (9)	45	70.5 (11)

* Number in parentheses gives reference; ages not referenced are estimated.

† R. L. Larson, personal communication.

Thus, reconstructions of the Indian and Eurasian plates, using marine magnetic anomalies and differences in the position of palaeomagnetic poles obtained from these plates together place constraints on the ages of both the anomalies and the samples studied palaeomagnetically. These results are consistent both with results of Sclater *et al.*[11] and Larson (personal communication), suggesting that anomaly 24 is younger than suggested by Heirtzler *et al.*[5], and with the idea that the Deccan Traps may be older than measured. The results presented here are also consistent with an age for anomaly 32 younger than that suggested by Heirtzler *et al.*[5]. Plate reconstructions are apparently sufficiently accurate to be used as a laboratory tool for age dating and 'stratigraphic' correlation over large distances.

One of us (J.F.) did this work while on leave from the Centre Océanologique de Bretagne in the Department of Earth and Planetary Sciences of MIT and thanks X. Le Pichon, F. Press, C. Riffaud and J. G. Sclater for providing this opportunity. This research was supported by the National Science Foundation.

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Received March 4; accepted March 18, 1975

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Mechanism for nucleation and growth of manganese nodules

FERROMANGANESE oxide crusts and concretions cover vast areas of the sea floor[1,2] and the relatively high concentrations of cobalt, nickel and copper in manganese nodules, together with the high population densities of polymetallic nodules on certain sea beds, such as the North Pacific Equatorial Belt[3], mean that these marine sediments are potential ore deposits. A fact that is often overlooked, however, is that in many sedimentary environments most of the manganese and iron does not accumulate in the form of ferromanganese nodules, but instead is distributed throughout the sediment in the form of dispersed microscopic concretions[4]. A fundamental problem in accounting for the origin and distribution of polymetallic nodules is to explain the nucleation and growth of the host ferromanganese oxide phases. We demonstrate here that crystal-structure relationships between host manganese (IV) oxide and iron (III) oxide hydroxide phases control the authigenesis, mineralogy and metal enrichments of manganese nodules.

Observations of the interiors of manganese nodules under the microscope[5], together with electron microprobe analyses[6–8], reveal the intimate associations and oscillatory intergrowths of the manganese- and iron-bearing minerals. Most nodules have formed about a nucleus consisting of pumice, altered basaltic rock or glass, clays, tuffaceous material, or hard parts of organisms (shark teeth, whale bones, globigerina tests, coral or radiolaria tests). Microprobe analyses of the boundaries between the nuclei and ferromanganese oxide layers[9,10] reveal that a hydrated iron oxide phase coats the nucleating agent before the cyclic deposition of Mn oxides plus trace metals and Fe oxyhydroxides occurs. The hydrated iron oxide substrate is probably precipitated at interfaces where the *p*H is locally high as a result of dissolution and hydrolysis of the carbonate, silicate and phosphate anions derived from the nucleating agent[10]. Such evidence for the growth of the Mn oxide phase on a hydrated Fe oxide substrate, together with the intimate association of the ferromanganese oxides across manganese nodules, suggests that crystallographic relationships exist between the constituent Mn and Fe oxide minerals.

Table 1 lists selected manganese (IV) oxide and iron (III) oxide hydroxide minerals for which crystal structure data are available. Todorokite ("10 Å manganite"), the crystal structure of which has not been determined, is also an important constituent of manganese nodules. The acicular habits of todorokite crystallites in terrestrial deposits[11] and in manganese nodules[12] suggest to us that todorokite may have a tunnel structure similar to that of hollandite or psilomelane. Several of the minerals listed in Table 1 are isostructural; thus, each of the pairs goethite and ramsdellite, akaganéite and the hollandite group, and probably $FeOOH.xH_2O$ and δ-MnO_2, have identical crystal structures or contain similar planes of atoms. In such isostructural minerals, oriented intergrowths or epitaxy of one crystalline phase on the other may occur, provided that dissimilarities of lattice parameters or interplanar spacings are not large. Indeed, epitaxial growths of ramsdellite ($a_0 = 4.53$ Å, $b_0 = 9.27$ Å, $c_0 = 2.87$ Å) on goethite ($a_0 = 4.65$ Å, $b_0 = 10.0$ Å, $c_0 = 3.04$ Å) are observed in geodes[13,14]. The mismatch of unit cell dimensions between these two minerals is less than 7%, and seems to be small enough to enable epitaxy between goethite and ramsdellite. The ramsdellite crystal structure[15] contains Mn atoms surrounded by six oxygens at the corners of an octahedron. The $[MnO_6]$ octahedra share edges with adjacent octahedra to form double chains which are cross linked to adjacent double chains by corner sharing of oxygen atoms forming structure with orthorhombic symmetry. In goethite, the double chains of edge-shared $[Fe(O,OH)_6]$ octahedra are further linked by hydrogen bonds[16]. Another way of viewing these two structures is in terms of a hexagonal close-packed oxygen framework in which Mn^{4+} or Fe^{3+} cations are distributed in an ordered array among the octahedral sites. The structure of hydrated ferric oxyhydroxide polymer is believed to contain Fe^{3+} ions in a hexagonal close-packed network[17,18], but the cations are randomly distributed in the octahedral sites with but random order among them. The phase δ-MnO_2 is considered to be a disordered birnessite[19,20] and to contain fragments of the layers of edge-shared $[MnO_6]$ octahedra with small periodicities of stacking along the *c* axis. Thus, δ-MnO_2 also consists of a network of hexagonal close-packed oxygens containing Mn^{4+} ions in the octahedral sites. The structural similarities between δ-MnO_2 and $FeOOH.xH_2O$ make them amenable to epitaxial intergrowths.

We conclude, therefore, that the nucleation and growth of many manganese nodules is initiated by the epitaxial growth of δ-MnO_2 on to the $FeOOH.xH_2O$ substrate coating the nucleating agent. Episodic growths of these two phases on one another lead to the intimate association of Mn and Fe minerals in manganese nodules. Such intergrowths of δ-MnO_2 and $FeOOH\ xH_2O$ inhibit the formation of the ordered layer

Table 1 Isostructural manganese and iron oxide minerals

Manganese (IV) oxides	Manganese (III) oxide hydroxides	Iron (III) oxide hydroxides	Structure type
Pyrolusite β-MnO_2	—	—	Rutile
Ramsdellite MnO_2	groutite α-MnOOH	*Goethite α-FeOOH	Diaspore
*Nsutite γ-MnO_2	—	—	Pyrolusite+ramsdellite
Hollandite group $(Ba,K,Pb,Na)_{1-2}Mn_8O_{16}.xH_2O$	—	*Akaganéite β-FeOOH	Hollandite
*Psilomelane $(Ba,K,Mn,Co)_2Mn_5O_{10}.xH_2O$	—	—	Psilomelane
—	—	*Lepidocrocite γ-FeOOH	Boehmite
—	—	Un-named δ-FeOOH	Disordered goethite
—	Feitknechtite β-MnOOH	—	Brucite
—	Manganite α-MnOOH	Un-named ε-FeOOH	Manganite
*Birnessite ("7 Å manganite") $(Ca,Na,Mn)Mn_7O_{14}.3H_2O$	—	—	Birnessite
*δ-MnO_2 ("protobirnessite")	—	*$FeOOH.xH_2O$ ("ferrihydrite") (hydrated iron (III) oxyhydroxide polymer)	Hexagonal close-packed oxygens
*Todorokite ("10 Å manganite") $(Na,Ca,Mn)Mn_3O_7.xH_2O$	—	—	Unknown

*Reported in manganese nodules.

structure of birnessite. We suggest, however, that post-depositional recrystallisation facilitates long range ordering and may produce periodic stacking sequences characteristic of the birnessite and todorokite structures, the todorokite phase being stabilised by high water pressures[10].

Research supported by the National Science Foundation—IDOE Ferromanganese Nodule Program.

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Received February 13, accepted April 1, 1975

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Hydrocarbons associated with lead–zinc ores at Laisvall, Sweden

BITUMEN and a series of normal alkanes (C_{18} to C_{27}) have been identified in sphalerite from the Laisvall lead–zinc deposit. This is a stratabound lead–zinc ore in Eocambrian sandstone, situated at the eastern border of the Swedish Caledonides. The deposit is an aberrant member of the Mississippi Valley ore class[1]. The main ore minerals are galena and sphalerite, associated with minor amounts of calcite, barite and fluorite. These infill interstices around the sandgrains, often following sedimentary structures[2]. The lead is markedly radiogenic J-type with a $^{206}Pb/^{204}Pb$ ratio of more than 20 (ref. 3). Fluid inclusion studies on the sphalerite give homogenisation temperatures around 150 °C (ref. 4) Laisvall is the largest lead concentration (original resources of about 60 × 10^6 t of 4% Pb) in the 1,000 km long lead- and zinc-rich Caledonian border zone, which includes deposits such as Dorotea and Vassbo.

Samples of the ore and enclosing rocks were taken both from drill cores and hand specimens from the mine itself. Crushed rock samples were washed and shaken three times for 24 h with acetone. The samples were further crushed to less than 63 μm with a pestle and mortar cleaned with acetone. Three grammes of the powder in 5 ml toluene were placed in an ultrasonic bath for 30 min, filtered and evaporated to dryness. The residue was dissolved in 0.10 ml ether–acetone (1: 1), and 3 μl of the solution was injected into a glass capillary column OV-1, 25 m long, in an LKB 2091 gas chromatograph–mass spectrometer–computer system.

The results are listed in Table 1. Total extractable organic matter was variable up to a maximum of 100 p.p m. In most cases the gas chromatogram consisted of a single broad peak, representing a complex mixture of high molecular weight isomers, which can be loosely described as bitumen. In two samples a series of alkanes were identified which made up about 10% of the total extractable material (Fig 1).

The extractable organic matter was concentrated only in those samples containing sphalerite. Samples rich in galena, silica, calcite, fluorite or barite contained low to negligible amounts of extractable material. Furthermore, all sphalerite samples examined were relatively rich in organic matter. We therefore conclude that the organic material is closely associated with the sphalerite and further, that the organic material is partly situated within the abundant[5] fluid inclusions in the sphalerite, although attempts to identify the location of the material (for example by ultraviolet microscopy) have not been successful to date.

Our evidence that this organic material is natural and not an artefact is based on several lines of reasoning. In particular: (1) the enclosing sandstone host rock associated

Table 1 Estimated contents of bitumen and normal alkanes from Laisvall specimens

Specimen no.	Composition	Source	Type	Organic material Estimated total concentration (p.p.m.)	Concentration of normal alkanes (p.p.m.)
L01X	Sphalerite*, calcite, quartz	Nadok orebody	Bitumen	100	Not determined
L02X	Galena*, calcite, quartz	Strand orebody	Bitumen	1	Not determined
L03X	Calcite*, galena, quartz	Lower sandstone (core)	Bitumen	Not detected	Not detected
L04X	Galena*, quartz	Kautsky orebody	Bitumen	1	Not determined
L05X	Galena*, quartz	Nadok orebody	Bitumen	1	Not determined
L06X	Sphalerite*, calcite, quartz	Nadok orebody	Bitumen	100	Not determined
L07X	Barite*, quartz	Lower sandstone (core)	Bitumen	1	Not determined
L08X	Fluorite*, quartz	Lower sandstone (core)	Bitumen	1	Not determined
L09X	Sphalerite*, calcite, silica	Nadok orebody	Bitumen+C_{18}–C_{27} Normal alkanes	~40	4
L11X	Sphalerite*, calcite, silica	Nadok orebody	Bitumen+C_{18}–C_{27} Normal alkanes	~40	4

The estimations were made by comparison with a C_{19} standard. For this reason the estimates of total organic material (as bitumen) are presented as orders of magnitudes, whereas the alkane concentrations are accurate to within ±1 p.p.m.
*Principal constituent.

with these samples, is essentially impermeable with a porosity approaching zero (M.W., unpublished); (2) similar samples, without sphalerite, contain low, possibly background, amounts of organic compounds; (3) both core and hand specimen samples were examined; (4) analyses of possible contaminating substances give either no measurable organic contamination (such as acetone) or different gas chromatograms (such as machine oil and grease); (5) the laboratory preparation was such that the samples could only be contaminated during the final crushing (that is, after acetone-washing of the preliminary crushed samples and before the ultrasonic extraction) and this stage was rigorously controlled; (6) although the possibility of airborne contaminating material dropping into the samples cannot be entirely precluded, the observations that only samples rich in sphalerite suffered this fate and even in these samples different gas chromatograms were obtained, seem to rule out even this possibility.

The identified alkanes in specimens L09X and L11X include the high molecular weight paraffins C_{18} to C_{27} (Fig. 1). A peak observed after C_{27} is consistent with squalene. Work is continuing on this material to identify the origin and authenticity of this observation. The carbon preference index (CPI) is very low, about 0.64. The absence of lower molecular weight alkanes and the low CPI with its even number preference indicates that these substances are not derived directly from biological materials. They must have developed as a result of secondary reactions from the original organic substances. This is not only further evidence against contamination of the samples by modern biological substances, but is also consistent with the age and probable history of the deposit. The sphalerite was formed before the

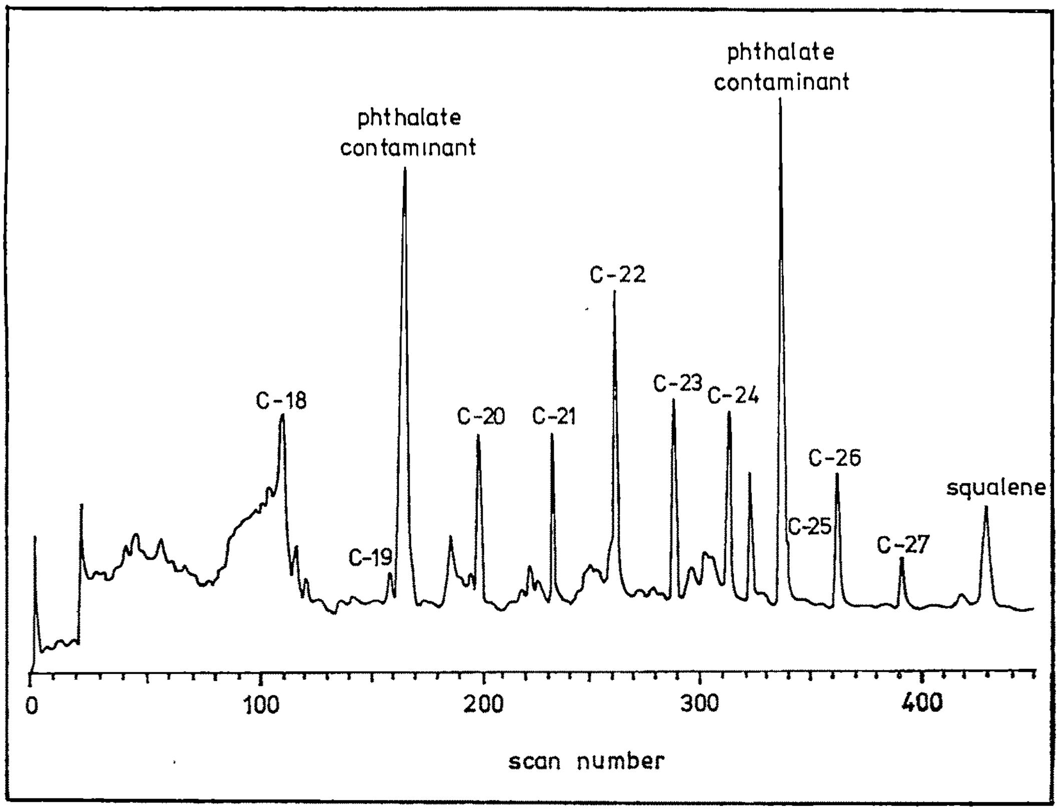

Fig. 1 Gas chromatogram of specimen L09X (sphalerite, calcite and quartz), showing a series of normal alkanes C_{18}–C_{27} and squalene. Glass capillary column OV-1, 25 m long, from 120 °C to 250 °C at 10 °C min^{-1}.

Caledonian orogeny and during or after the Eocambrian sedimentation, a period of about 200 Myr commencing about 600 Myr BP.

It has been proposed on the basis of isotope studies, that the ore forming fluids involved in the formation of Mississippi Valley-type ores were highly saline oilfield brines[1]. The evidence from our study adds further weight to this hypothesis since, in the case of Laisvall, it seems that the solutions contained petroleum-type compounds.

This work was supported by the Swedish Natural Science Research Council and the Boliden Company.

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Received March 17; accepted April 1, 1975.

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Experimental alteration of chlorites into vermiculites by chemical oxidation

VERMICULITE and chlorite minerals occur commonly in soils where they may be an important source of magnesium and micronutrients. Vermiculite adsorbs and retains potassium and ammonium preferentially, which makes it particularly important in exchange reactions involving these cations. Like micas, chlorites in soils may weather to vermiculites[1,2]. Micas are readily altered to vermiculites in the laboratory by the extraction of potassium. It has not, however, been possible to alter unoxidised, true chlorites to vermiculites by extracting the hydroxide sheets using chemical techniques[3,4] in the laboratory.

I demonstrate here that pure, unoxidised chlorites (IIb polytype) containing an appreciable amount of ferrous iron may be changed to vermiculites in the laboratory using a simple and effective chemical oxidation technique. I show the importance of the oxidation of structural ferrous iron in this alteration process and describe some properties of the vermiculite products.

The alteration technique consists of reacting chlorites in saturated bromine water on a steambath. Samples of 300 mg of 2–5 μm cholite were placed in screw cap, Pyrex vials containing 20 ml of saturated bromine water. The vials were capped tightly and placed in the steambath. After the appropriate time the vials were opened and the bromine boiled off. The samples were separated by centrifugation and then treated twice with sodium dithionite[5] to remove free iron-hydrous and aluminium-hydrous oxides. Portions of the samples were taken before and after the oxidation and dithionite treatments and decomposed in HF (ref. 6). The silicon content was determined colorimetrically[8] and Fe, Al and Mg were determined by atomic absorption spectrophotometry. Ferrous iron was also measured[7]. X-ray diffraction patterns were obtained from specimens basally oriented on glass slides using an instrument with Fe-filtered, Co radiation.

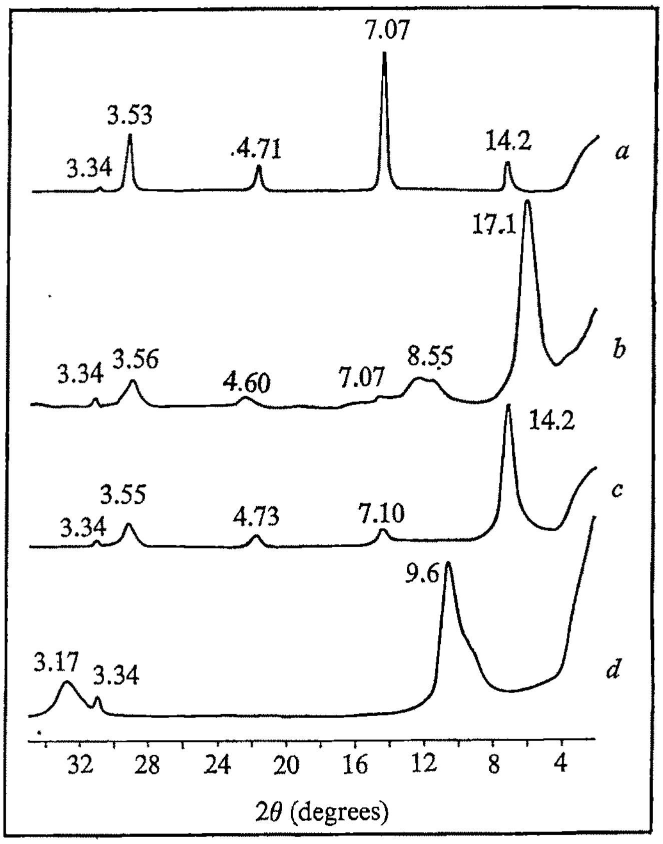


Fig. 1 X-ray diffraction patterns of a high-iron chlorite (diabantite from New Mexico). *a*, Original sample; *b*, *c* and *d*, after 3 weeks' reaction: *b*, saturated with Ca and glycerol; *c*, saturated with Mg and glycerol; *d*, saturated with Ca and heated at 700 °C. Spacings in Å.

Figure 1 shows the transformation of high-iron chlorite — $(Al_{1.57}Fe^{3+}_{0.28}Fe^{2+}_{2.89}Mg_{0.68})(Si_{3.32}Al_{0.68})O_{10}(OH)_8$—into vermiculite after three weeks of reaction. The vermiculite product expands to 17.1 Å when saturated with Ca and glycerol (Fig. 1*b*) and to 14.2 Å when saturated with Mg and glycerol (Fig. 1*c*). This expansion behaviour resembles that of low-charge vermiculites with a cation exchange capacity (CEC) of around 130m equivalent per 100 g. Heating at 700 °C contracts the layers to 9.6 Å but a shoulder at the low-angle side of this peak indicates some (~20%) remaining chlorite layers. The CEC increased from 1.8 to 100.4 meq per 100 g during the reaction.

A low-iron chlorite— $(Al_{1.00}Fe^{3+}_{0.04}Fe^{+2}_{0.39}Mg_{4.72})(Si_{2.62}Al_{1.38})$-$O_{10}(OH)_8$—when treated in the same way as the high-iron chlorite showed very little alteration to vermiculite even after 5 months of reaction (see Fig. 2*a*, *b* and *c*).

The evidence (Figs 1 and 2) suggests that the oxidation of structural ferrous iron is an important mechanism in the alteration of chlorite to vermiculite. This is further brought out by the data in Table 1 which show that the reaction in acid solution without addition of an oxidising agent has little effect on chemical composition and CEC. The X-ray diffraction pattern of this sample also remained identical to that of the original chlorite. In the presence of bromine as an oxidising agent, the composition and CEC change drastically, reflecting the alteration of chlorite to vermiculite. The X-ray diffraction patterns of this sample are shown in Fig. 1*b*, *c* and *d*.

Assuming that the Si-to-Al ratio of the tetrahedral sheets does not change and that 20% of the original chlorite remains unaltered, the formula of the vermiculite portion (80%) in the altered product is $(Al_{0.44}Fe^{3+}_{1.19}Fe^{2+}_{0.40}Mg_{0.15})(Si_{3.32}Al_{0.68})O_{10}(OH)_2\,[Ca_{0.34}]^{0.68+}$. The number of cations (Al, Fe and Mg) in the octahedral sheet is 2.18 which shows that there was a considerable loss of these cations from the octahedral sheets of the mica-like layers during the alteration process. This was also indicated by a decrease in the

Table 1 Partial chemical analysis of original and altered chlorite (diabantite from New Mexico)*

Cation	Original	Original plus dithionite treatment	Three weeks reaction in HCl (*p*H 2.0)† followed by dithionite treatment	Three weeks reaction in Br_2 water (*p*H 1.8)‡ followed by dithionite treatment
Al	8.93	8.93	9.50	5.97
Fe^{2+}	23.70	25.56	24.10	7.84
Fe^{3+}	2.30	0.44	1.82	9.58
Mg	2.44	2.69	2.62	1.20
CEC (m equivalent per 100 g§)	1.8	6.9	13.1	100.4

*Based on sample weight at 110 °C.
†Initial *p*H 1.4; final *p*H 2.0.
‡Initial *p*H 2.2; final *p*H 1.8.
§Ca exchanged with Mg.

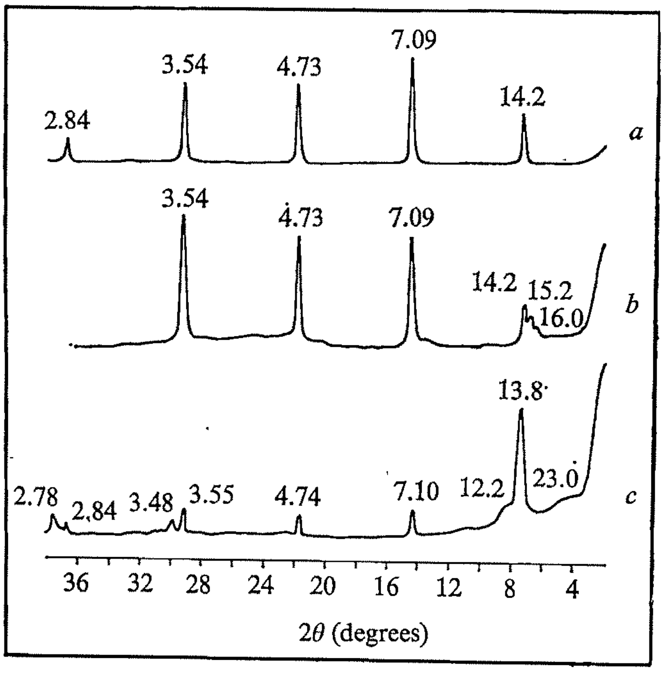
d(060) spacing from 1.557 Å in the original chlorite to 1.537 Å in the altered product, which comprised approximately 20% chlorite layers. Decreases in the *d*(060) spacings because of losses of octahedral cations have also been reported in the oxidation of biotite[8].

It has been proposed previously that selective dissolution of the hydroxide sheet is induced by a structural disturbance of this sheet[9]. During chemical oxidation this structural disturbance is probably caused by the oxidation of ferrous iron and its ejection from the hydroxide sheet.

The results reported here indicate that the oxidation and removal of ferrous iron play an important role in the weathering of chlorites to vermiculites in soils. This was also suggested in a study which showed that one extraction by sodium sulphite or sodium dithionite of a partially oxidised iron chlorite resulted in the formation of a regularly interstratified chlorite-vermiculite[2]. The abundance of chelating agents present in most soils would facilitate the removal of iron from the chlorite structure under less acid conditions than those used in my experiment.

As well as providing clarification about chlorite weathering in soils, the ability to change chlorites to vermiculites in the laboratory provides the opportunity for comparison between the structure and behaviour of chlorite-derived vermiculites and mica-derived vermiculites; it is, however, impossible to do this with soil vermiculites as they are mixed inextricably. Of particular interest in this comparison would be the exchange behaviour with potassium and ammonium, which could well be influenced by the origin of the vermiculites[10].

Fig. 2 X-ray diffraction patterns of a low-iron chlorite (sheridanite from Brewster, New York). *a*, Original sample; *b* and *c*, after 5 months' reactions at *p*H 2.0: *b*, saturated with Ca and glycerol; *c*, saturated with Ca and heated at 700 °C. Spacings in Å.



I thank M Jaakkimainen and N. M. Miles for their technical help and Drs H. Kodama and J. D. Adshead for reviewing the manuscript.

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Received February 7, accepted March 25, 1975.

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Chemical transport by the Mekong river

Major rivers are important for an understanding of the overall balance of dissolved and particulate matter carried to the ocean[1,2]. Although Livingstone[3] gives a large amount of information on the world surface waters, there is still a lack of data on many important rivers, especially from South-east Asia. These rivers are generally presumed to contribute a great part to the continental and particulate balance[1,3,4], but are poorly documented except for the work of Kobayashi in Thailand[5] and a few analyses performed for the International Association of Scientific Hydrology[6] The Mekong river is one of the biggest in this region[7,8] (Table 1) We have previously studied the hydrology of the Tonle Sap-Mekong system[9] and the influence of the Mekong river on Cambodia Grand Lac water chemistry[10]. Here we deal with analyses performed at Phnom Penh (Fig. 1).

At this station the basin area is 663,000 km^2, 83% of the total drainage area, and mean annual discharge is 14,900 m^3 s^{-1}. Between January 1961 and December 1962, 546 daily samples were taken in which temperature, *p*H and conductivity were measured. The river was at that time affected little by war activities. Once a month a complete water analysis was performed. In this report we set up the mean annual water quality value, compare transport rates

Table 1 Largest rivers of the world according to the water and dissolved salt discharges

River	Water discharge $m^3\ s^{-1}$	Basin area $10^3\ km^2$	Salt discharge $10^6\ t\ yr^{-1}$	Reference
Amazon	175,000	6,300	290	14
Congo	39,200	4,000	50	19
Orinoco	30,000	950	50	3
Yang Tse	22,000	1,950	≃100*	
Mekong	21,200	795	60	—
Brahmaputra	19,200	580	≃ 80	—
Mississippi	18,400	3,267	142	15
Parana	18,000	2,800	57	18
Yenissei	17,200	2,600	≃ 73†	1, 13
Lena	16,300	2,430	≃ 85†	1, 13
Ganges	11,610	990	76	—
Danube	6,430	805	60	16
Mackenzie	9,600	1,800	60	17

*Minimum value, on the basis of a 50 t $km^{-2}\ yr^{-1}$ transport rate.
†Total ionic transport plus 10% for silica transport.

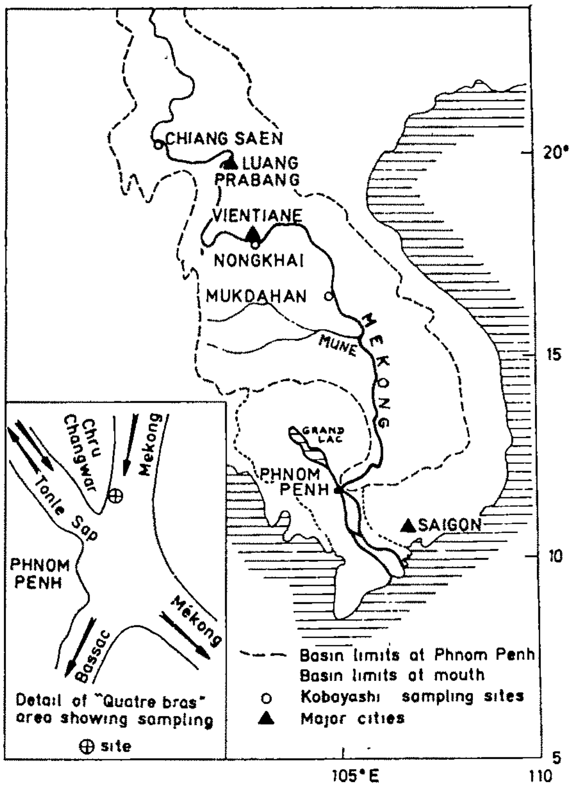

Fig. 1 Map of area.

at Phnom Penh with upstream rates in Thailand recomputed from Kobayashi values and compare chemical erosion in the Mekong basin with those of other Himalayan rivers and determine the importance of South-east Asia for the global dissolved balance.

Mean daily total ionic contents (Σ^{+-} in mg l^{-1}) have been computed on the basis of daily conductivities measured at 25 °C (measured in μmho cm^{-1}) converted into Σ^{+-} according to the relationship

$$\Sigma^{+-} = 0.72 \times \text{conductivity} + 14.3$$

(regression computed for 23 values, $r = 0.97$). The total ionic transport for 1961–62 is $45.5 \times 10^6\ t\ yr^{-1}$. Since the water discharge during this period was very close to the mean annual discharges (14,970 $m^3\ s^{-1}$ as against 14,900 $m^3\ s^{-1}$), this value can be taken as a fair average. Mean major element contents have been computed on the basis of the 24 instantaneous dissolved discharges corresponding to monthly analyses. Total ionic transport given by this less accurate method is $42.6 \times 10^6\ t\ yr^{-1}$ so that annual transport averages for each element were corrected. As dissolved silica is not influencing conductivity, mean SiO_2 transport was not corrected (Table 2). As for the other big rivers[1–3], bicarbonate and calcium are the principal ions carried by the Mekong. The proportion of silica (8.5%) is similar to Livingstone's world mean[3] (11%).

We have recomputed discharge-weighted concentration averages at three Thailand stations studied by Kobayashi[5]: Chieng Saen, Nongkai and Mukdahan (Fig. 1 and Table 1). We used drainage area values and discharge measurements at Mukdahan[11] for estimating monthly discharges at Chieng Saen and Nongkai that were not available to Kobayashi. Transport rates at Chieng Saen are characteristic of the mountainous upper Mekong basin. The total mineral transport rate (86 t $km^{-2}\ yr^{-1}$) is relatively low as compared to other high-relief basins such as the Ucayali river in the Andes[2] (152 t $km^{-2}\ yr^{-1}$), or the Rhone river (France) at mouth (190 t $km^{-2}\ yr^{-1}$). The transport rates are nearly constant along the 1,850-km river course from Chieng Saen to Phnom Penh. The lower Mekong basin is chiefly exposed to monsoon rains and the relief is much more gentle. The

Table 2 Quality variation along the Mekong river

Location	SiO_2	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	SO_4^{2-}	HCO_3^-	NO_3^-	Total ionic	Total mineral
Chieng Saen*† 195,000 km²‡ 1,955 km§	6.9	13.8	2.3	2.9	0.72	1.9	5.8	51.1	0.04	78.5	85.4
Nongkai*† 310,000 km² 2,650 km	7.1	13.0	2.1	2.5	0.75	1.9	4.3	49.3	0.08	73.9	81.0
Mukdahan*† 390,000 km² 3,250 km	8.2	13.7	2.1	2.9	0.77	1.9	3.8	51.7	0.11	77.0	85.2
Phnom Penh† 663,000 km²	6.3	10.1	2.2	2.6	1.4	7.5	2.7	41.2	0.82	68.5	74.8
3,800 km‖	8.85	14.2	3.2	3.6	2.0	10.5	3.8	57.9	1.1	96.5	105.1

*Recomputed from data of Kobayashi[5].
†Transport rate t $km^{-2}\ yr^{-1}$.
‡Drainage area.
§Distance from source.
‖Concentration in mg l^{-1}.

Table 3 Dissolved transport rate of Mekong compared with other Himalayan rivers

		SiO_2	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	SO_4^{2-}	HCO_3^-	NO_3^-	Total ionic	Total mineral
Mekong	(a)	6.3	10.1	2.2	2.6	1.4	7.5	2.7	41.2	0.82	68.5	74.8
	(b)	5.0	8.0	1.75	2.05	1.1	5.95	2.15	32.8	0.65	54.5	59.5
Ganges	(a)	3.8	9.4	3.0	4.1	1.3	3.2	4.1	48.7	0.5	74.3	78.1
	(b)	3.7	9.15	2.9	4.0	1.3	3.1	4.0	47.5	0.5	72.5	76.2
Brahmaputra	(a)		21.2	3.6	3.1	2.4	5.2		90			
	(b)		12.3	2.1	1.8	1.4	3.0		52			
Mean of Himalayan rivers	(a)	4.9	12.8	2.9	3.4	1.65	5.2	3.5	57.4	0.7	87.6	92.5
World mean	(c)	4.2*	4.9†	1.2†	1.65‡	0.55‡	2.3†	4.2†	16.6†		31.4	35.6

(a) Transport rate (t km^{-2} yr^{-1})
(b) Contribution the Ocean ($10^6 \times$ t yr^{-1})
(c) For the continent surface contributing to the ocean balance, 101×10^6 km^2, glaciated area excluded.
*Recomputed from Livingstone[3],
†Recomputed from Alekin[1] and Brazhnikova[13].
‡Na + K = 2.15 t km^{-2} yr^{-1} from refs 1 and 13, K ≃ Na/3 according to Livingstone[3].

total transport rate in this region can be estimated as 75 t km^{-2} yr^{-1}, still two times the world average (see Table 3). Monsoon action over mid-altitude relief is as erosive as cold climate action over high mountains of the upper basin. The order of transported elements is the same from Chieng Saen to Phnom Penh apart from an increase of Cl^- between Mukdahan and Phnom Penh that can be explained by high Cl^- input between these two stations from Thailand tributaries such as the river Mune[5].

The Mekong dissolved transport rates are compared to Ganges and Brahmaputra rates in Table 3. The Ganges value was computed from three single analyses quoted by Raymahashay[12] at Farakka, by Livingstone[3] at Calcutta and given by G. K. Seth (unpublished). For the Brahmaputra we found only a single reliable analysis at Pandu[12]. So no discharge-weighting has been carried out and these water quality values are inaccurate to the extent of ±50% error for SO_4^{2-} and NO_3^-. General characteristics for these rivers were taken from the UN-ECAFE commission[7,8]. The Brahmaputra transport rates are by far the highest. Total transport is up to 145 t km^{-2} yr^{-1}, the maximum for any such river, and it can be accounted for by the very high relief of this basin (60% is within the Himalaya range compared with only 25% for the Ganges and the Mekong rivers) and by precipitation that is responsible for a high specific discharge, 36 l s^{-1} km^{-2}. Relief is, for Brahmaputra, the main environmental factor that controls dissolved transport; this influence has also been noted for the Amazon basin[3] and for rivers in the Soviet Union[1,13,14]. Ganges transport rates are very similar to the Mekong ones, which may be caused by the similarity of the topographic and climatic features of their basins.

If the transport rates determined at Phnom Penh are extrapolated to the remaining basin area (we assume that there are no major changes arising from rice paddy fields in the Delta), the Mekong carries 1.7% of the global dissolved mineral input to the ocean ($3,600 \times 10^6$ t yr^{-1}, Table 3). This gives it eighth position in the dissolved input 'league' (Table 1). This classification is somewhat different from that of Gibbs[20]. The dissolved matter carried by the 10 'top' rivers is around 33% of the total input to the ocean. We have estimated the dissolved transport rate for mid-relief basins exposed to the monsoon as 75 t km^{-2} yr^{-1}. If this value is taken as a first estimate for South-east Asian rivers still not investigated in detail (Yang Tse Kiang, Pearl, Irrawady, Salween . . .) and for the Indonesia and New Guinea archipelagos rivers, the contribution of South-east Asia from the Ganges to the Yang Tse Kiang to the dissolved discharge to oceans may be more than 30%. It seems necessary, therefore, to study further the geochemical processes in these regions.

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Received February 10; accepted March 25, 1975.

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A fifteen-year record of biotic metabolism in the Northern Hemisphere

THE health and productivity of the biosphere are essential to man's welfare. Several scientists have speculated that global productivity and/or biomass should be increasing because of such factors as plant fertilisation from industrially derived atmospheric CO_2 enhancement[1,2]. Others have maintained that global primary production and biomass must be decreasing because of pollution effects, such as from acid rain, and forest cutting[3,4]. There are some (rather controversial) tree-ring data that may support this view[5,6]. If one or the other of these trends is true, there would be a shift in the absolute and relative rates of global photosynthesis, respiration and their ratio (the PR ratio). Earlier attempts to ascertain any such trends from long term changes in atmospheric CO_2 concentration have been frustrated by difficulties in distinguishing changes in biospheric retention from changes in the uptake or discharge of other reservoirs, principally the sea and industrial sources[7,8]. We present here a new model for the examination of such trends that we believe avoids such problems by focusing on potential changes in yearly patterns of atmospheric CO_2.

A nearly continuous record of the atmospheric concentration of CO_2 has been maintained for the past 16 yr at Mauna Loa, Hawaii (19.5°N, 155.6°W, 3,400 m altitude[7,9,10]) which is located within the well-mixed tradewind belt. The annual variation in CO_2 content there is 6 p.p.m., roughly representative of the Northern Hemisphere, where the annual variation ranges from about 3 p.p.m. at 10°N to a measured maximum of about 15 p.p.m. at Point Barrow, Alaska (71°N ref. 11). The Mauna Loa data provide the only suitable intensive atmospheric CO_2 record available for the Northern Hemisphere over a sufficient length of time.

These data, which are remarkably smooth and consistent, were obtained with a well calibrated, continuously recording gas analyser, and are corrected for local effects as described by Keeling and coworkers[9,10]. Data are unavailable for only six of the 178 months of this record, and values for these months are estimated from the least square fit of the data to a function of time which included oscillatory and secular terms[7,10].

We, and others[12-14], attribute the seasonal variation in atmospheric CO_2 (about 6 p.p.m.) principally to seasonal changes in net photosynthesis and respiration of the biosphere, although purely physical and chemical criteria, such as annual variations in sea surface temperatures, may contribute slightly. The secular increase (about 0.8 p.p.m. yr^{-1}) is attributed to atmospheric retention of a fraction of the CO_2 produced by industrial processes (principally the combustion of fossil fuels, although the kilning of limestone and other anthropogenic activities contribute a small fraction). Nearly all industrial fuel burning occurs in the Northern Hemisphere.

Our procedure for the analysis of net hemispheric metabolism require the normalisation of the month-by-month CO_2 data by subtracting the industrially derived CO_2. Since month-by-month global industrial fuel combustion numbers are not available, we estimate this by multiplying the global annual rates of anthropogenic CO_2 production by monthly proportions determined from patterns[15-17] in the USA (Fig. 1*b*). These numbers are then corrected for the estimated rate of oceanic CO_2 uptake. The resulting curve, which would be the month-by-month pattern if there were no industrial additions during this time, is plotted in Fig. 1*c*.

We base our oceanic uptake corrections on the work of Broecker, who has estimated that as of 1970 the partitioning of industrial CO_2 among atmospheric and oceanic reservoirs is such that $58 \pm 7\%$ remains in the atmosphere[18]. His calculation is based on a model of the world's oceans which includes a thin, warm, mixed surface layer separated from the cold, deep ocean by a 1 km thick principal thermocline. His model also accounts for enhanced exchange of atmospheric carbon with the deep oceans in the cold, turbulent high latitudes.

Annual terrestrial net photosynthesis for each year is determined by subtracting the trough of each normalised annual curve from the preceding crest. Respiration is determined by subtracting each trough from the following crest. There is no direct precedent for determining net photosynthesis and respiration by this technique, although the method has conceptual forerunners in daily and seasonal net photosynthesis and respiration techniques well developed in the aquatic sciences and used occasionally in forest metabolism studies. The analysis would give us 'net ecosystem production' and 'net ecosystem respiration' as defined by Woodwell and Whittaker[19], although they did not explicitly consider these definitions in terms of annual net growing and non-growing seasons, respectively, as we do.

Therefore we call our measurements 'net hemispheric metabolism,' which includes 'semiannual net photosynthesis' and 'semiannual net respiration.'

A model of hemispheric metabolism that would give the observed curves (Fig. 1*c*) is shown in Fig. 2*a*. In a simulation of global metabolism, using twin sine waves as in Fig. 2*a*, we found our analysis to be sensitive to fairly small increases or decreases in photosynthesis (defined as above) or similar changes in respiration (Fig. 2*b*), but simultaneous unidirectional changes in both photosynthesis and respiration were difficult to discern. But, as discussed below, we would not expect simultaneous unidirectional changes to occur. So we believe this analysis valid for discerning global metabolic trends.

We did not observe any significant trend in either "semiannual net photosynthesis' or 'semiannual net respiration' for the period of this analysis (Fig. 1*d*). Linear regressions of both the photosynthesis (P) and the respiration (R) values were compared to a line of zero slope using Student's *t* tests. Neither regression line was different from the horizontal at the 50% level. Additionally, the slope of the linear regressions for the 15-yr trends in both photosynthesis and respiration, after correction for 51, 58 and 65% atmospheric retention of industrially derived CO_2, never exceeded the standard error of the estimate of the slope for any of these lines.

We do, however, observe an increase in the PR ratio from 1962 through 1968, a change that would produce the dip seen in the calculated data of Fig. 2*c*. Since this change is not explicable by water temperature changes, we assume that this period was relatively favourable to photosynthesis and/or unfavourable to respiration. There was also some slackening in world food

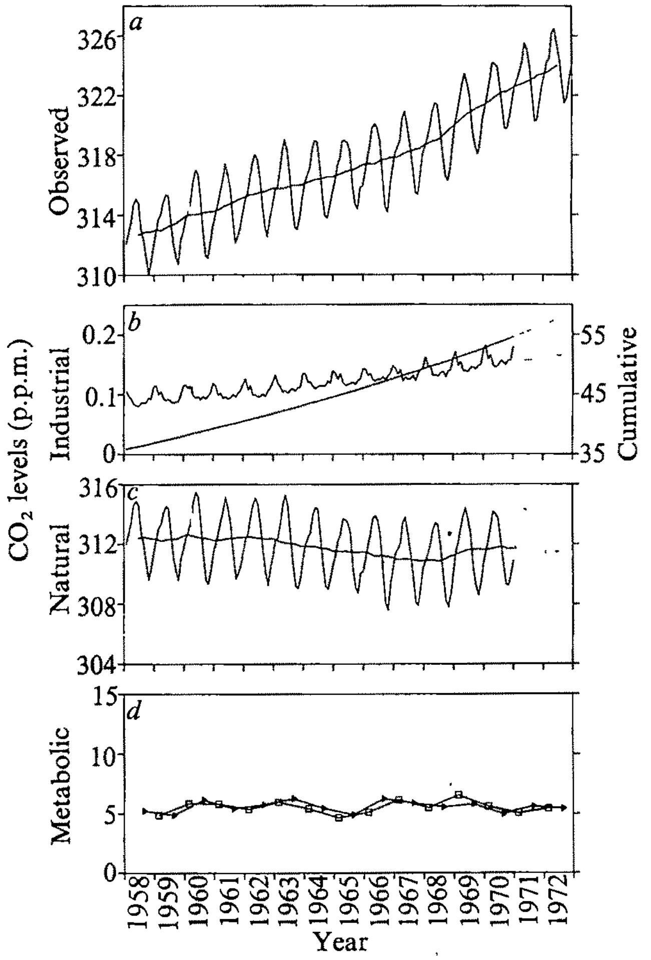

Fig. 1 *a*, Month-by-month variation in the CO_2 concentration of tradewind air at Mauna Loa, Hawaii. The upward sloping smooth curve is normalised for the semiannual fluctuations. *b*, The month-by-month addition of industrial CO_2 to the Northern Hemisphere, along with the cumulative production above the preindustrial level of 293 p.p.m. (ref. 25). The past two years are extrapolated values from the least square fit of data to a third-order polynomial. *c*, The observed CO_2 variation corrected for net industrial additions. *d*, Semiannual net ecosystem production and respiration, determined as the difference between the crests and troughs, in Fig. 2*c*. □, Respiration by difference; △, photosynthesis by difference. Oceanic uptake 42%.

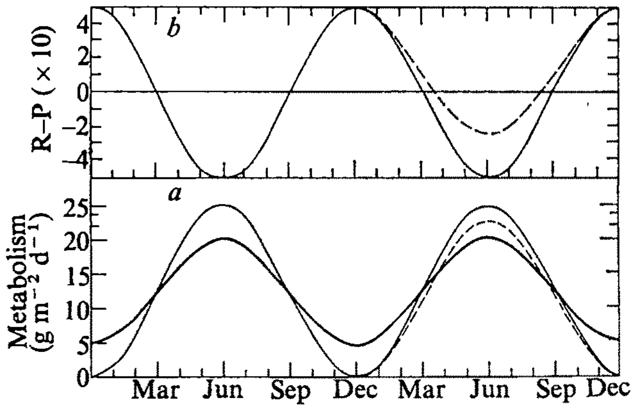

Fig. 2 *a*, A model of terrestrial biospheric metabolism extrapolated from the behaviour of the Brookhaven oak–pine forest[12,26,27]. The difference between 24-h respiration (heavy line) and gross photosynthesis (lighter line) would produce the CO_2 fluctuations plotted as the solid line in Fig. 2*b* or Fig. 1*c*. A 10% reduction in gross photosynthesis would produce the patterns shown as dotted lines in Figs 2*a* and 2*b* superimposed on an upward secular trend as less CO_2 is removed from the air each year.

production during 1964 and 1965, although in general such annual correlations between our estimates of photosynthesis and world food production are not very strong.

There are several possible sources of error in our analysis. The most important include: an incorrect coefficient for the uptake of CO_2 by the sea; an underestimate of the quantity of CO_2 injected into the atmosphere by man's activities; and possible effects of annual or secular trends in oceanic surface temperature. We investigated each in turn by sensitivity analysis of our calculations and/or extensive calculations of work presented previously[13,20-22]. The results of these analyses (available from C.A.S.H.) strengthen our belief in the relative insensitivity of our principal conclusions to possible errors in the data base, and confirm our confidence in the numbers of Keeling, Rotty and Broecker.

Although there are several other factors that could interfere with our analysis that are not well understood at present or not measured, such as annual differences in Hadley cell or other atmospheric circulation patterns, we would expect any secular changes in metabolism to emerge above these, and show as a consistent increase or decrease in one of the lines in Fig. 1*d*, or in their relative positions. For example, a decrease in photosynthesis over a period of years probably would be accompanied by a decrease in the PR ratio, as decompositional processes continued to break down forest biomass. Similarly, if man were cutting forests significantly relative to net photosynthesis, the trees would be oxidised by way of decompositional pathways or burning, and the PR ratio would decrease. This is not consistently the case.

Therefore, we tentatively conclude that, although industrial processes have altered and often degraded many aspects of the biosphere. there is no evidence for changes in the basic pattern of Northern Hemisphere biotic metabolism from 1959 to 1972 within the limits of resolution by this method. Either the biosphere is too big to be affected yet or the potentially adverse effect of such things as acid rain, atmospheric dust loading, urbanisation or replacement of highly productive forests with less productive agriculture is approximately balanced by the stimulatory effects of atmospheric CO_2 enrichment and the increased use of fertilisers in agriculture[3]. In addition, the community compensatory mechanisms elucidated by Botkin and his colleagues[24] in computer simulations of forests would tend to damp potential changes. But if man's impact continues to grow at an exponential rate it will be interesting to follow the gross effects on the biosphere. We suggest our method and its future refinements as one such global index, and recommend that other CO_2 monitoring stations be established at different latitudes and altitudes to facilitate this.

Part of this research was carried out at Brookhaven National Laboratory under the auspices of the US Atomic Energy Commission. The collection of the CO_2 data was performed under the auspices of the National Science Foundation. We thank D. Juers for statistical assistance, R. Houghton for ideas on forest metabolism, C. Cogbill for thoughts on tree-ring analysis, A. Hermann for help with the simulation and H. Lieth, R. H. Whittaker and G. M. Woodwell for critical comments.

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Volcanic influence on seawater at Heimaey

VOLCANISM is a contributing factor in marine geochemical processes. It has been shown to contribute to the dissolved silicon in seawater[1], it has also been suggested as the cause of some anomalous fluoride concentrations observed in the sea[2] and it has been seen as a possible source of manganese, iron and other elements found in unusual concentrations in manganese nodules and some deep sea sediments[3,4]. Moreover, it has been suggested that in the geological past volcanism has affected the microfauna of large oceanic areas[5] Here I describe the effect of the recent Heimaey eruption on the waters nearby.

The eruption on Heimaey, the largest of the Westman Islands, started on January 23, 1973, and lasted about five

Table 1 Chemical properties of surface seawater adjacent to the Heimaey lava

Sample no.	Date (1973)	T (°C)	S (‰)	SiO_4-Si (μg-atom l^{-1})	NO_3-N (μg-atom l^{-1})	F/Cl* ($\times 10^5$)	Mn (μg l^{-1})	Fe (μg l^{-1})	Zn (μg l^{-1})	Hg (ng l^{-1})	F (mg kg^{-1})
V 1	10.2		35.948	99.0	10.3	14.2	177		28.2		2.827
V 2	,,		38.268	236	6.9	22.6	482		47.4		4.791
1	9.3	7.3	35.054	14.8	13.9	6.97	20.9	129	7.0	109	1.352
2	,,	6.7	35.063	12.2	14.3	6.90	15.9	82.8	12.4	70	1.340
3	,,	6.7	35.043	12.2	14.5	6.86	11.9	50.4	5.8		1.331
4	,,	8.3	35.102	21.6	14.2	7.18	35.6	111	5.4	111	1.395
5	,,	15	36.097	88.0	12.9	8.89	199	86.4	13.7	161	1.776
6	,,	8.0	35.137	24.3	14.2	7.21	32.8	28.8	4.2		1.403
7	,,	16	36.210	113	12.7	9.21	243	79.2	14.5		1.847
8	,,	19	36.672	115	12.1	10.1	300	83.5	18.3	305	2.058
9	,,	10.5	35.120	42.5	13.8	7.70	75.8	68.4	6.8		1.497
10	,,	19	35.361	88.0	12.4	9.84	139	41.0	13.1	256	1.927
11	,,	23	35.506	115	11.5	10.8	181	43.2	14.6	364	2.120
12	,,	11	35.079	23.1	14.0	7.15	24.9	36.7	13.5	478	1.389
13	,,	24	35.374	95.5	10.8	8.70	98.5	28.8	21.6	78	1.703
14	,,	31	35.419	119	9.1	9.27	123	35.3	15.4	166	1.817
15	,,	10.5	35.002	22.9	14.1	7.27	26.6	32.4	22.3	37	1.408
16	,,	17	35.449	75.0	12.5	8.65	101	21.6	26.6	320	1.698
17	,,	21	35.513	86.0	11.5	8.93	112	13.0	17.3	239	1.756
18	,,	21	35.346	86.0	11.3	8.57	84.9	21.6	14.3	196	1.676
19	,,	12.2	35.090	29.7	13.7	7.27	32.3	45.3	22.2	439	1.413
20	,,	16	35.256	64.0	12.7	8.32	79.2	32.4	17.3		1.624
21	,,	17	35.275	68.0	12.7	8.77	96.8	43.2	12.0	134	1.712
22	,,	10.4	35.138	49.0	13.6	7.70	85.5	87.9	19.9		1.497
23	,,	6.50	35.051	10.8	14.6	6.81	5.0				1.322
24	,,	6.21	35.051	9.2	14.6	6.83	5.0				1.325
A 1	30.3	23	36.339	163		10.4	183	22.3	14.7	10.0	2.102
A 2	,,	29	38.272	401		14.1	589	11.5	13.2	9.0	2.990
A 3	,,	11.0	35.105	56.0		7.72	37.9	57.6	15.7	6.7	1.500
A 4	,,	19	35.138	73.2		10.0	79.8	301	18.2	9.7	1.953
A 5	,,	13.0	35.153	66.7		8.68	63.9	166	15.3	5.1	1.690
A 6	,,	8.0	35.039	34.8		7.38	50.4	61.9	17.9	10.0	1.431

V1 and V2 samples, small volumes, collected from shore on the harbour side of the advancing lava. Samples 1–24 (Fig. 1) and A1 to A6 were collected from RV Bjarni Saemundsson and from a rubber dinghy by filling 4l polyethylene containers by immersion. The samples were subsequently split into subsamples. Trace metal samples, except mercury, were acidified with redistilled HCl. Mercury samples were collected from the rubber dinghy. Samples A1 to A6 came from the sheltered region off the northern lava front.

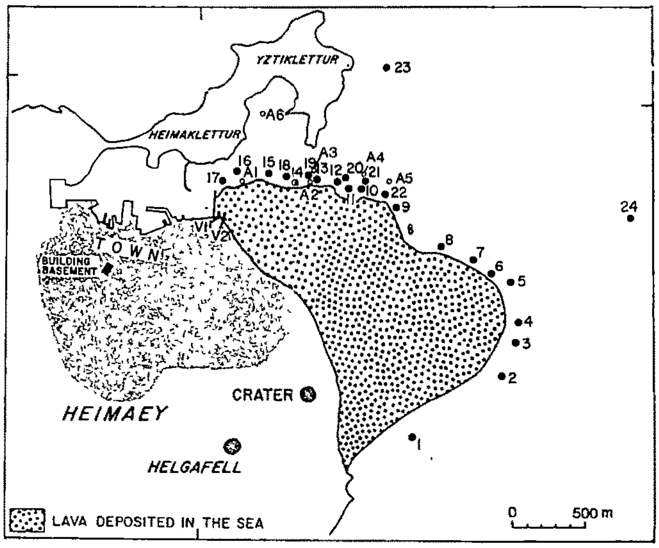
* Cl = S/1.80655.

months, during which a substantial fraction of the volcanics was deposited in the sea[6]. Although the eruption did not occur under the sea it provided an opportunity to observe mobilisation of elements which resulted from the interaction between seawater and volcanics. The material for this study is listed in Table 1. In addition, the extent of the area affected and the normal regional conditions were assessed by examination of samples collected on February 10 during an oceanographic survey of RV Bjarni Saemundsson in the region of the Westman Islands. Temperature and salinity were determined as well as concentrations of fluoride[7], silicate[8], nitrate[8], manganese[9], iron[10], mercury[11], zinc and copper[12].

At Heimaey, the influence of volcanism on the adjacent seawater arose mainly from the viscous lava that advanced spasmodically into the sea. This caused the temperature to rise and also, by evaporation, increased the salinity from the regional range of S=35.0‰ to 35.15‰. On March 9 the warm surface layer that spread out from the lava front was generally thin with a thickness rarely exceeding 1 m. As might be expected this warm surface layer contained a concentration of dissolved silicon considerably in excess of the concentrations of 9–10 μg-atom Si l^{-1} found in the unaffected water.

The solubility and rate of dissolution of silicon in water increases with temperature[13,1] and there is a significant positive correlation (r=0.77) between dissolved silicon and temperature (Table 1) and an even stronger correlation between dissolved silicon and salinity (r=0.91). Since the increased silicon concentration is the result of a dissolution process at the seawater–lava interface, it is of interest to relate the other constituents examined to the concentration of dissolved silicon. All samples with significantly increased dissolved silicon concentrations also had enhanced fluoride/chlorinity ratio and there is a significant correlation (r=0.75) between the two parameters. This confirms that volcanism may considerably increase the fluoride/chlorinity ratio of seawater, although it is clear that such anomalies are unlikely near quiescent volcanoes[14,15]. The fluoride added to seawater in the eruption may either be derived directly

Fig. 1 Seaward position of lava front and sampling locations on March 9, 1973. ▲, Samples collected February 10; ●, samples collected March 9; samples collected March 30.



from the gaseous emanations or be leached from the surface of the lava and tephra. A 100 mg l^{-1} fluoride solution has been obtained by leaching lava ejected on the first day of the Heimaey eruption[16], and the close correlation between dissolved silicon and the fluoride/chlorinity ratio suggests that fluoride has been introduced chiefly by leaching.

The manganese concentration close to the lava front (Table 1) was much greater than the regional background concentration of 2–5 μg l^{-1}; it shows a strong correlation (r=0.94) with dissolved silicon (Fig. 2*a*). Comparison with rock analyses of the new Heimaey lava[16], shows that whereas the elemental ratio by weight of manganese to silicon is 0.011 in the lava, the elements go into seawater solution in the ratio 0.058; this must leave the parent material impoverished in manganese. The distribution pattern of iron around the lava front is altogether different from that observed for manganese and there is no correlation with dissolved silicon or other enriched components. The greatest silicon and manganese concentrations and a very low iron concentration were found in a sample (A2) of seawater that had percolated through a lava front that had been stationary for some time but still remained warm. This suggests that manganese is more readily leached from the lava than iron. The highest iron concentrations on March 9 were associated with brownish patches observed in the sea; this was also the case on March 30. No attempt was made to separate colloidal size particles from samples and in the acidified conditions used for storage, colloidal ferric hydroxides will have redissolved. The distribution of iron suggests that the iron (II) leached from the volcanics was rapidly oxidised and that the colloidal hydroxides have partially separated from the enriched solution, probably by wave action, to create the patchy appearance. A differential precipitation mechanism of this type, that separates iron and manganese, has been anticipated[17] and is thought to be the cause of the distribution of these metals in some deep sea sediments[3,18].

Thermal and redox conditions at the seawater–lava interface are important in the mobilisation of silicon, manganese and iron. For silicon the temperature is of greatest importance, but manganese and iron will only go into solution in reducing conditions. Iron in the fresh Heimaey lava has been shown to be rapidly oxidised in an atmospheric environment[16]. The greatest dissolution of iron and manganese would therefore be expected to occur when the lava front advances into the sea continually exposing new surfaces to attack by seawater. The importance of redox conditions was borne out in a simple experiment where tephra that fell on the RV Bjarni Saemundsson on March 9, 1973, was leached with seawater (1 g per 200 ml) for 15 min using a boiling water bath, the resultant solution gained 41 μg-atom Si l^{-1}, 6 μg Mn l^{-1} and 4 μg Fe l^{-1}, but in a parallel experiment where the solution was 10^{-3} M with respect to hydroxylammonium chloride and slightly reducing, the gains were 41 μg-atom Si l^{-1}, 11 μg Mn l^{-1} and 31 μg Fe l^{-1}.

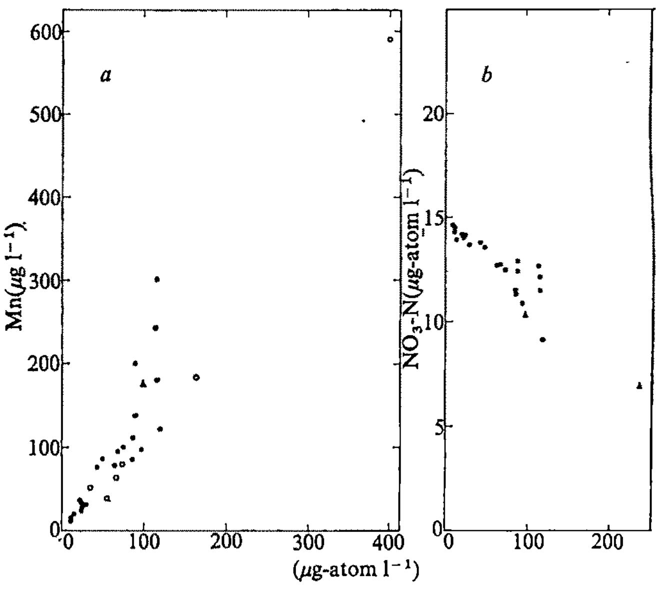


Fig. 2 Relationship between dissolved silicon and: *a*, manganese, *b*, nitrate nitrogen in the seawater near the lava front. ▲, Samples collected February 10; ●, samples collected March 9; ○, samples collected March 30.

Table 2 Determinations of mercury in gas samples

Date	Sample	Volume sampled (l)	Mercury* (μg m^{-3})
October 2, 1974	Reykjavík, air†	540	0.007
March 29, 1973	Heimaey town, street air	10	0.44
March 28, 1973	Heimaey town, building basement gas	1.2	3.34
		2.4	3.70
	Fumarole on south-west side of the crater, volcanic gas	1.2	15.0
		1.2	19.0
April 26, 1973	Chimney on lava lake, north east of the crater, volcanic gas	1.0	7.91
		1.0	10.8
		0.5	16.1

*Refers to volume of non-condensing gases.

†Collected by amalgamation on gold. Mercury in all other samples collected by drawing the gas sample with associated condensed water through silicon tube into a gas washing bottle containing 50 ml of 2% potassium permanganate in 25% sulphuric acid.

The zinc concentration was relatively high in many of the samples enriched in silicon; however, there was no significant correlation between the two, which shows that zinc is not brought into solution in proportion to the silicon dissolved. Determinations of copper, cobalt and nickel were also carried out, but only in one instance was an increase above the normal background level observed; this was for sample A2 in which 16 μg Cu l^{-1} was found—about three times the background.

The nitrate concentration decreases near the lava front with an inverse correlation to dissolved silicon, $r=-0.93$ (Fig. 2*b*). This somewhat unexpected finding might be explained as being the result of reduction of nitrate nitrogen to molecular nitrogen (or nitrous oxide) under the reducing conditions at the seawater–lava interface.

The extent of the volcanic influences on the chemistry of seawater was limited to the immediate neighbourhood of the eruption. The water temperature inside the harbour, which was initially 6.5 °C did not rise above 25 °C during the first four days of the eruption. During this time the northward extension of the eruptive fissure was active in the sea[6]. Samples from the oceanographic survey on February 10 revealed anomalies only at three stations that were at a distance between 2.2 and 4.6 km from the eruption. The anomalies were in temperature (up to 10.5 °C), dissolved silicon (26.6 μg-atom l^{-1}), manganese (28 μg l^{-1}) and the fluoride/chlorinity ratio which attained a maximum 7.15×10^{-5}, compared with $(6.79\pm0.07)\times10^{-5}$, for unaffected samples from this survey which is close to the normal oceanic ratio[19] of 6.67×10^{-5}.

The mercury concentrations found near the lava front on March 9 (Table 1) were high for south Icelandic coastal water[11] and the highest concentrations do not correlate with either temperature or dissolved silicon. While collecting these samples it was twice observed that gas bubbles rose to the sea surface off the northern stretch of the lava front. Three weeks later, when the force of the eruption was waning, no gas bubbles were noticed and the samples collected had low mercury concentrations (Table 1). Considering that the seawater mercury may be derived from the rising bubbles of volcanic gas, an effort was made to determine the mercury concentration in the latter. The results summarised in Table 2 show that in comparison with the normal air[20] from Reykjavík, the Heimaey values are high. On April 26 sampling conditions were particularly favourable near the crater and I believe that the gas then sampled represents the unmodified volcanic gas, but I am not certain whether this applies to the fumarole gas. The gas that issued into building basements and lower parts of the town was highly modified, usually containing more than 90% CO_2 (ref. 6). No comparable data are available for the concentrations of mercury in the gaseous emanations of erupting volcanoes, but geothermal mobilisation of the element is well known[21]. It is tempting to use the results in Table 2 to estimate the total amount of mercury ejected into the atmosphere in the eruption. For this purpose it is necessary to assume that the amount of both mercury and water vapour evolved during the eruption was proportional to the amount of solid ejecta, $250\times10^6\,m^3$, or about 600×10^6 t (ref. 6). Second, from solubility data[22], an upper limit of 8% by weight is assumed for the water content of the magma that erupted from a depth of about 20 km (ref. 6). The most representative volcanic gas samples collected during the Heimaey eruption had an average water mole fraction of 63% (Bragi Árnason, personal communication). From the above and the average mercury concentration of 14 $\mu g\ m^{-3}$ in the lava lake and fumarole gases, the upper limit for gaseous mercury emission in the eruption is 7×10^5 g. This is a small quantity in comparison with the anthropogenic atmospheric input which has been estimated at $3\times10^9\ g\ yr^{-1}$ from coal combustion alone[23].

I thank all those who helped in the collection of water and gas samples.

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Received January 30; accepted March 26, 1975.

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Recognition of the oldest known fossil marsupials from Australia

A PRELIMINARY study has been carried out on fossil remains (unearthed in the British Museum) from a travertine deposit in Tasmania. Regardless of the precise taxonomic assignments ultimately given to this material, the evidence we present that a diverse fauna of diprotodont marsupials existed in Australia in late Oligocene time is of considerable importance. This evidence gives tangible support to the hypothesis that marsupials have been residents of the Australian continent since the early Tertiary at least. The basic differentiation of herbivorous Diprotodonta from Marsupicarnivora (Ride, 1964) very likely took place before the separation of Australia from Antarctica. By late Oligocene time Tasmania was situated near 52°S latitude[1] bathed by warmer seas[2] and the travertine accumulated in an equable warm-temperate to subtropical environment supporting a rich forest vegetation[3-5] the closest living equivalents of which occur in the uplands of New Guinea and New Caledonia. Present-day representatives of some of the fossil marsupials from the travertine still inhabit such tropical environments in northern Australia and New Guinea.

Allport[6] has reported the discovery of fossil mammal remains in the travertine at Geilston Bay (42°50′S, 147°21′E) on the northern shore of the Derwent across the estuary from Hobart, Tasmania. At that time the travertine outcrops at the head of the Bay had been commercially quarried for over 20 years and were well known to naturalists for their rich content of fossil plant and animal remains. Allport noted that the bones we exhibited included forms similar to those of living potoroos and phalangers. Owen's report[7] that "fragments of bones, some teeth, and ungual phalanges of a small kind of *Hypsiprymnus*, with probably also *Perameles* and *Phalangista*" had been identified, led Allport[8] to state that the "bones all proved to belong to existing species", a conclusion that necessitated a considerable revision of the geological history postulated for the site. Allport was forced to explain how the bones of living species could be found many feet below the ground surface and beneath a basalt flow interbedded in travertine associated with a fossil flora having little in common with the present-day flora of Tasmania. Nothing more was written about the bones until Johnston[9] intimated that "it is to be feared they have not been preserved". The Geilston Bay collection and its whereabouts thus passed into obscurity primarily because of the belief that it represented only a Recent fauna.

Mahoney, conducting a search for bibliographic materials relating to the Australian fossil mammals in the British Museum (Natural History), located the Allport collection in the museum's holdings. Preliminary examination of the fragmentary remains leaves no doubt that the fossils represent new taxa of marsupials. An assemblage of Tertiary age is strongly indicated, and this recognition has led us to re-examine the geology of the Geilston Travertine and associated basalts to determine the age of the fossiliferous deposit.

The bones were found[6] in an "arenaceous clay, containing coarse grit, and a few slightly rounded pebbles" interbedded with the travertine. Matrix still adhering to some specimens confirms this description of the containing rocks. Allport also indicated that work west of the quarry towards the River Derwent had exposed the east dipping contact of the travertine with the overlying basalt. Several geologists have observed the quarry working face, for travertine was produced from the site at least until 1924. Johnston[10] recorded (in descending order) the following composite section in the quarry and adjacent area: 1.5 m of weathered basalt overlying 1.2 m of silicified ostracod limestone, 1.8–2.4 m of yellow and brown mottled calcareous clay with marl lenses from which he alleged the marsupial remains were obtained, 3.1–3.6 m of travertine with plants and snails and 1.8 m or more of brown clay extending below the level of the estuary. A similar section, but without the basalt, was recorded by Krause[11] in his map of part of the Hobart area and Nye[12] reported "thin layers of limestone occur above the basalt" as well as beneath. Moore[13] gives one of the last direct reports on the abandoned quarry and maps two outcrops of basalt along the south side of Geilston Bay, both of which are shown to extend to the edge of the estuary. Between these outcrops Moore's map shows "hornblende tuff", but this is a printing error, for the outcrops in that position are the Geilston Travertine (W. R. Moore, personal communication). Our observations show that the westernmost basalt outcrop overlaps Permian (Malbina) siltstone at a higher elevation than shown on Moore's map, and that it extended beyond the head of the bay to become continuous with the eastern outcrop, and to thus overlap the Geilston Travertine. These relationships seem to indicate that the basalt partially filled a narrow canyon containing a spring which had built up a considerable travertine apron before being buried by basalt and which subsequently continued to deposit travertine above the basalt. The combined observations of many geologists extending over 100 yr clearly indicates that the Geilston Travertine is mostly sub-basaltic (that containing the marsupial bones certainly so) and that travertine deposition was halted for a time by the basalt incursion and it is thus reasonably contemporaneous with the basalt. These conclusions had been anticipated[14].

It is now impossible (because of development) to secure a sample of the basalt at the travertine quarry. Therefore samples of the basalt from the western outcrop were collected for possible isotopic dating, and the least altered of these (Tasmanian Museum No. 22241; ANU No. 74-98) was measured by the K–Ar method. The sample was collected approximately 100 m from the head of Geilston Bay at sea level on the southern shore. This basalt contains about 5% mainly fresh olivine phenocrysts set in a well-crystallised fine-grained groundmass of plagioclase, clinopyroxene, olivine and iron oxide, together with 5% green mineraloid and minor calcite. The plagioclase shows evidence of some low temperature deuteric alteration. The basalt yielded an apparent age of 22.4 ± 0.5 Myr ($K=0.765\%$; rad. $^{40}Ar=3.07\times10^{-11}$ mol g^{-1}; 100 rad. ^{40}Ar/total $^{40}Ar=90.0$; $\lambda_e=0.585\times10^{-10}$ yr^{-1}; $\lambda_\beta=4.72\times10^{-10}$ yr^{-1}; $^{40}K/K=1.19\times10^{-2}$ atom %). Because of the somewhat altered nature of the basalt the measured age must be regarded as a minimum estimate for the time of crystallisation because of the possibility of some diffusive loss of radiogenic argon. The measured age approximates closely the estimated age of 22.5 Myr for the Oligocene–Miocene boundary[15], thus the Geilston Travertine underlying the basalt is considered to be late Oligocene or older.

The oldest previously known Australian marsupial *Wynyardia bassiana* was also from Tasmania at Fossil Bluff on the north-west coast where a skeleton was recovered from the Fossil Bluff Sandstone of early Longfordian age[16]. Recent radiometric calibration of the Longfordian Stage from the northern side of the Bass Basin in Victoria suggests that its base is approximately 21.4 Myr (ref. 17) and thus *Wynyardia* is of early Miocene age.

At least three mammalian taxa can be discerned among the very fragmentary remains from the Geilston Travertine. All identifiable remains seem referable to the Order Diprotodonta (Owen, 1866) and none can be relegated to the Peramelidae (Waterhouse, 1838) as Owen had stated. The largest form is represented by a fragment of a left maxillary with well worn dentition including part of M^1 and M^2–M^4 complete (BMNH 40157, length M^2–M^4 25.1 mm). This dentition resembles the Miocene palorchestine diprotodontid *Ngapakaldia* (Stirton, 1967) closely enough to encourage tentative assignment to that subfamily. It is about 60% of the size of *N. tedfordi* (Stirton, 1967) from the Ngapakaldi Fauna of the Lake Eyre Basin. More abundantly represented is a second and smaller form that is postulated on the basis of tentatively associated edentulous jaw fragments, an unworn left M^1 (BMNH 32000, length 4.3 mm) and left M^2 or M^3 (BMNH 32001, length 4.2 mm), and other tooth and skeletal fragments. In mandibular dental formula and morphology these remains resemble those of the smaller species of *Phalanger* (Storr, 1780) and although other features bar assignment to that genus, reference to the Family Phalangeridae (Thomas, 1885) seems likely. A third form is indicated by a lower incisor which agrees best with those of living Burramyidae (Broom, 1898).

We thank J. A. Mahoney (University of Sydney) and the trustees of the British Museum (Natural History) for permission to report on the Geilston fauna, and W. R. Moore for data on the geology of the site. This research was supported in part by a NSF grant to R.H.T.

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Use of a self-made sound baffle by a tree cricket

FIELD observations and sound recordings of several South African species of Oecanthidae were carried out during the summer and autumn of 1973–74 to augment studies of the acoustic behaviour of the tree cricket performed abroad[1–6]. We report that *Oecanthus burmeisteri* uses a leaf as a sound baffle to increase the intensity of its calling song by pressing its tegmina against the edges of a pear-shaped hole gnawed into the leaf.. At least two more South African chirping *Oecanthus* species use a leaf baffle just as efficiently as *O. burmeisteri.*

The calling song of the male *Oecanthus burmeisteri* Saussure consists of regular chirps repeated for hours on end during the summer, from dusk to about 2 h past midnight. The chirping sound is actually produced by a frictional mechanism involving the elytra. The right front wing bears the stridulatory file, a row of regularly spaced 'teeth'. A sclerotised edge of the left wing serves as the scraper. When the file and scraper are rapidly rubbed together a sound is produced. Chirps recur at about 2 s intervals, each complete chirp lasting for 300 ms and consisting of ten bursts, each burst being made up of an almost sinusoidal oscillation at 2,000 Hz. This frequency is near the lower end of the scale for *Oecanthus* calls.

Interest in the sound production of the Oecanthidae was aroused by the first *O. burmeisteri* male found singing on a small sunflower leaf on December 21, 1973 in Pretoria The insect producing the soft, insistent call was found on the underside of a leaf sitting in a small pear-shaped hole which it had gnawed and which differed from the irregularly shaped feeding holes The foremost part of its body

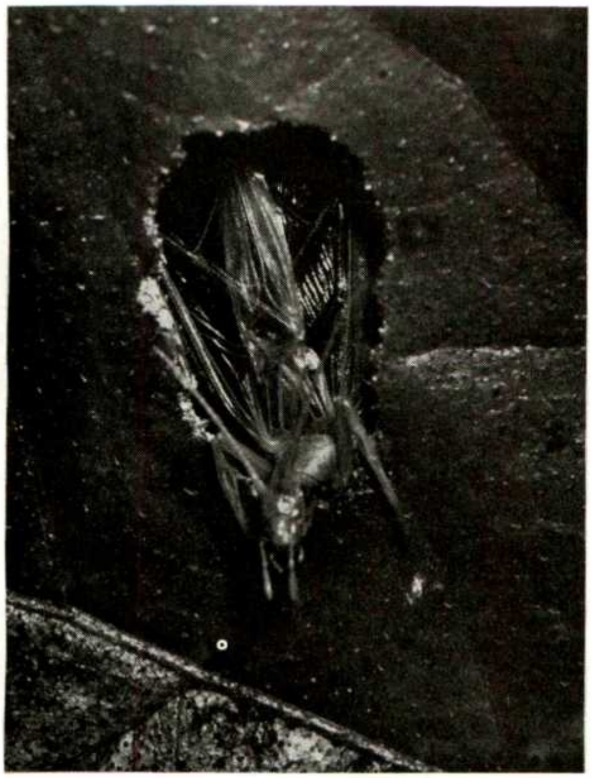
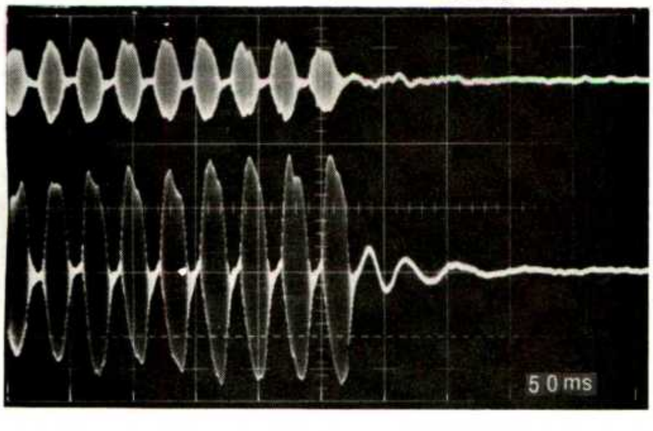


Fig. 1 Leaf used as a baffle. Wings pressed tightly against the edges of the hole gnawed by the insect.

and front legs protruded on to the upper leaf surface. During stridulation the elytra (or modified front wings) were raised at right angles and their lateral and apical margins were tightly pressed against the leaf surface surrounding the hole. Almost all male singers were sitting in similar positions.

Remembering that, according to Bennet-Clark[7], the mole cricket forms its burrow into the shape of twin exponential horns to increase its sound intensity, it occurred to us that *O. burmeisteri* may be fashioning and using a leaf for the same purpose. A microphone was therefore placed at a fixed position 15 cm from a male and a recording made first of the insect's song when sitting in front of the hole and then immediately afterwards when this individual was sitting inside the hole with its wings pressed against the leaf (Fig. 1). The orientation of the insect with respect to the microphone was the same in both cases. Consequently there was no possibility that directional effects in the sound radiation pattern, as suggested by Bennet-Clark[8], were present. Gain settings of the recording and reproducing systems were held constant.

Fig. 2 Oscillograms of two chirps of *Oecanthus burmeisteri*; upper trace, without baffle; lower trace, using baffle.



Oscillograms of the results obtained (Fig. 2) show that the sound amplitude is far greater in the second case, thus proving the effectiveness of the leaf baffle in amplifying the sound produced by stridulation. At the beginning of the chirp shown the amplitude ratio is 2.5 but rises to 3.5 for the final tone burst. This build-up is probably caused by the cricket steadily increasing the pressure of its elytra against the leaf, thus gradually increasing the effectiveness of the baffle.

A vibrating body, small compared with the wavelength of the sound it produces, is a poor sound radiator because a compression wave created on one side largely cancels the simultaneous rarefaction wave on the opposite side. For this reason Bennet-Clark[8] as well as Michelsen and Nocke[9] concluded that insects producing low frequency calls could increase their sound intensity by using a baffle. They show that as the size of a baffle placed around a sound source is gradually increased so the radiated sound intensity increases, reaching a maximum when the baffle diameter is approximately half the wavelength of the sound concerned. This is a well known acoustic phenomenon[10].

The wavelength of the 2,000 Hz note of *O. burmeisteri* is 170 mm, the diameter of its sound-producing membrane approximately 3.2 mm—only one-fiftieth of a wavelength. Consequently the membrane is a poor sound radiator. The sizes of the leaves used ranged from 70×80 to 170×300 mm, so even the smallest formed an efficient baffle. The hole, usually near the centre of the leaf where it would be most effective, averaged 8×14 mm in size thus ensuring a good fit for the elytra.

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Received December 11, 1974; accepted March 27, 1975.

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Measuring the relative importance of different pollinators to plants

THE development of a predictive theory of pollinator–plant coevolution requires a means of assessing and comparing quantitatively the costs and benefits of different pollinator foraging strategies and plant flowering strategies. Although there are quantitative data for pollinator strategies[1–3], the relative benefits to the plant of alternative pollinators, in terms of pollen transferred, ovules fertilised, and seeds set, have never been quantified for field conditions. Likewise, reference to the most effective pollinator[4] of a plant species is often based on estimates of the abundance of alternative pollinators. Demonstrations that various pollinators can cause seed set in caged plants[5] and that pollinators transfer pollen in different amounts[6,7] are difficult to relate to natural conditions. We have developed a quantitative approach for comparing the relative importance of alternative pollinators in terms of the quantity, level of out-crossing and efficiency of pollen transferred. We have used this approach to investigate the pollination of evening primroses, *Oenothera fruticosa* L. (Onagraceae), by two principal pollinators, European honey bees, *Apis mellifera* L., and soldier beetles, *Chauliognathus marginatus* Fab.

The quantity of pollen transferred per unit time can be calculated as the mean number of grains transferred to the stigma of a flower per pollinator visit multiplied by the mean number of flowers visited per unit time. Likewise, the quantity of pollen transferred per unit time by one species of pollinator can be calculated as the number of foraging individuals of that species multiplied by the quantity of pollen transferred by an individual per unit time.

Pollen transferred within a plant produces no seeds in self-incompatible species, and may result in lower quality seeds in self-compatible species. The proportion of pollen grains transferred that are out-cross (between plant) pollen (P) can be calculated considering the number of flowers visited per plant (N)[8,9], the number of pollen grains transferred per visit (G), and the number of grains on the body of the pollinator (B):

$$P = (1/N)\sum_{i=1}^{N}(1-G/B)^{N-1} \quad (1)$$

We assume that the pollen load on the pollinator is in dynamic equilibrium and that an equal number of pollen grains is exchanged on each visit.

The relative importance of soldier beetles and honey bees in the pollination of *O. fruticosa*, a self-incompatible plant, was investigated in a moist field north of Durham during May and June 1973 and 1974. The yellow flowers with eight stamens and a prominent, four-lobed stigma, remain open for 1 or 2 d (between approximately 0900 and 1700). Bees visited the flowers primarily for nectar, while beetles foraged for both nectar and pollen. Bees averaged 4.0 s per flower and beetles averaged 27.6 s per flower, during 341 and 54 visits, respectively. Over this time, the bees visited 4.9 flowers per plant whereas the beetles visited 2.6 flowers per plant. Bees and beetles were caught as they left the flowers and pollen was removed from them in agitated alcohol rinses. The bees averaged 1,100 pollen grains on their bodies ($n = 12$; s.d. $= 494$; range 436–1730) while the beetles averaged 217 ($n = 8$; s.d. $= 190$; range 19–570).

To estimate the pollen grains transferred per visit, virgin flowers were presented to the foraging bees and beetles and the grains on the stigma were counted after one visit. Bees transferred a mean of 193 pollen grains per visit ($n = 24$; s.d. $= 135$; range 9–548), while beetles transferred 114 grains per visit. The latter figure is based on only two observations, but for illustrative purposes the data for beetle pollen transfer will be used in further calculations. Individual bees and beetles transferred 174,000 and 14,900 pollen grains per hour, respectively. If the number of foraging bees and beetles is equal, then the former account for 92.1% of all pollen transferred. This estimate is biased in favour of beetles, as there seemed to be many more bees than beetles in any given area of the *Oenothera* population.

The level of out-crossing effected by the pollinators can be calculated from equation (1). For bees carrying 1,100 pollen grains, transferring 193 grains per visit, and visiting 4.9 flowers per plant, 71.2% of the pollen transferred would be out-cross pollen. For beetles carrying 217 grains, transferring 114 grains per visit, and visiting 2.6 flowers per plant, 63.5% of the pollen transferred would be out-cross pollen. Thus, bees would account for 92.9% of the out-cross pollen being transferred.

The energetic efficiency of pollen transfer was contrasted for the two pollinators on the basis of quantity of pollen transferred per calorie of nectar available. *O. fruticosa* flowers produced 0.3 µl of nectar at 45.3% sucrose equivalence or 0.52 calories per flower averaged across the population. Assuming that the pollinator removed all nectar from the flower during a visit, bees transferred 373 pollen grains per calorie of nectar produced, and the beetles 220 pollen grains per calorie. With a mean of 143 ovules produced per plant and a seed set of 54% averaged across the population, nectar production can be expressed as 0.036 calories of nectar per ovule produced or 0.067 calories per seed set. Individual bees transferred more pollen grains per unit time, more efficiently, than the beetles and a greater percentage of those pollen grains was out-cross pollen resulting in higher seed set. On the other hand, beetles may be able to forage profitably on smaller plants or poorer quality flowers because of their low level of activity and presumed lower caloric requirements. In sparse populations of *O. fruticosa* or on days when only a few flowers are open, beetles may increase in their relative importance as pollinators.

Differences in the pollination service provided by various pollinators may be an important selective force operating on plant populations. If pollinator service limits seed set, genotypes that possess flowers more attractive to a certain pollinator or a more efficient pollen attaching or removing mechanism will produce more seeds[10]. Alternatively, if pollinators are abundant, genotypes which receive the same pollinator service per flower while producing less pollinator attractants per flower, may be able to produce more flowers and set more seeds. Evolutionary shifts in floral morphology, colour and timing of flower production[11] are almost certainly associated with a new pollinator providing a higher quantity of pollen transfer or a higher level of out-crossing than a previous pollinator. The new pollinator may also provide the same level of pollen transfer but require less food during foraging. The honey bee as an introduced pollinator may impose a high selection pressure on *O. fruticosa* in populations where it is the primary and most efficient pollinator. Although insufficient time may have elapsed from introduction for the honey bee to have had an impact as a selective force on the particular set of characters that *O. fruticosa* now possesses, a responsive shift in floral characters may be predicted in the future. Only by measuring both the importance of pollinators to plants and the importance of plants to the pollinators, can the reciprocal selective forces which operate at the animal–plant interface be determined.

We thank F. Almeda, J. Antonovics, M. Berenbaum, B. Heinrich and D. E. Stone for suggestions. The study site was made available with the permission of John Blue. The authors were supported as NIH predoctoral trainees by a US Public Health Service training grant.

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Received February 10; revised March 28, 1975.

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^{14}C-ethylene incorporation and metabolism in pea seedlings

ETHYLENE is now generally accepted as an important, integral part of the natural hormonal complement of many plants[1]. Although the morphological, physiological and biochemical effects of the gas are well known, surprisingly little is known about its metabolism. There is no agreement as to whether or not ethylene is even incorporated into plant tissues. Several reports[2-4] of work using ^{14}C or ^{3}H-labelled ethylene indicate that it is not incorporated whereas others[5-10] suggest that a small, yet significant amount is fixed and metabolised. The criticism[1,11,12] of these papers, on the basis that adequate precautions were not taken to insure that the label fixed and metabolised was derived from ethylene, has led Abeles[1] to conclude that ethylene incorporation is probably not a prerequisite for ethylene action. A second major criticism of these studies is that no precautions were taken to exclude microbial contamination which could have accounted for the observed metabolism. Here, I report the results of experiments in which ^{14}C-labelled ethylene of very high purity was applied to etiolated pea seedlings under aseptic conditions to study its possible incorporation and metabolism. They reveal a previously unrecognised active metabolic system in pea which converts $^{14}C_2H_4$ to $^{14}CO_2$, as well as to unidentified tissue components.

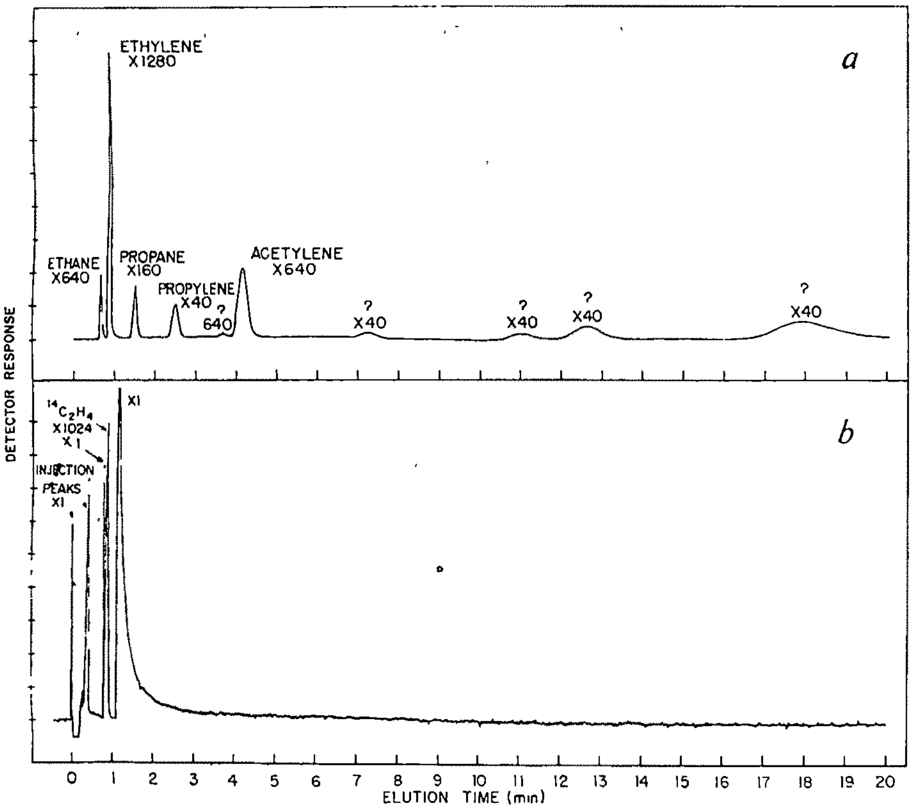

Fig. 1 Gas chromatograms of $^{14}C_2H_4$ (specific activity 53.0 mCi mmol^{-1}) before (*a*) and after (*b*) purification. Samples analysed on an activated alumina column using standard flame ionisation gas chromatography. This system could detect 6 parts per 10^9 C_2H_4 in a 1 cm^3 sample. At least nine major impurities were detected in the purchased $^{14}C_2H_4$ before purification whereas none was present in the twice purified material. The two $^{14}C_2H_4$ peaks at retention times of 0.80 and 0.85 min are the result of attenuating the $^{14}C_2H_4$ peak at 0.80 min from maximum sensitivity (×1) to ×1,024 to prevent it from going off scale. The apparent peak at 1.1 min is the result of increasing the sensitivity back to ×1 and represents the tailing of the $^{14}C_2H_4$ peak at this higher sensitivity.

Uniformly labelled $^{14}C_2H_4$ (reported radiochemical purity 98%, 22 or 53 mCi mmol^{-1}) was purified twice by gas chromatography (GC) and first separated on a silver nitrate ethylene glycol-coated Gas Chrom R column[11] at 0 °C. The ethylene fraction was collected and further purified using the same technique on a Poropak T column. Figure 1 illustrates the effectiveness of this purification scheme. No impurities were detected in the purified $^{14}C_2H_4$ even at maximum GC sensitivity.

With a gas-tight syringe fitted with a Millipore filter (0.22 μm), the desired amount of purified $^{14}C_2H_4$ was immediately injected into flasks containing three aseptically grown etiolated pea seedlings (0–5 d old). Each flask contained 3 ml sterile deionised water and 1 ml 1.5 N NaOH (electrolytic pellets) in the centre well. The flasks were placed on a shaker for 24 h in the dark at 27 °C. All operations were conducted using a 'safe' green light. Following incubation the ethylene concentration in each flask was determined and the seedlings plus the 3 ml of water were transferred aseptically to a mortar and homogenised. An aliquot (0.1 ml), diluted 20-fold, was plated on each of four different culture media to check for microbial contamination. Petri dishes were incubated at 30 °C for 1 week and the data from any sample showing contamination were not used. The remaining homogenised tissue was lyophilised, weighed, resuspended in water and counted by liquid scintillation in Aquasol (New England Nuclear). The NaOH was removed and 0.8 ml was counted in a similar manner. All data were corrected for counting efficiency and sample quenching by the internal standard method using ^{14}C-hexadecane.

A highly significant and readily detectable amount of $^{14}C_2H_4$ incorporation and conversion to $^{14}CO_2$ was observed in pea seedlings when treated with 15 μl l^{-1} of $^{14}C_2H_4$ (Fig. 2*a*). During the first 20 h after imbibition, the amount of $^{14}C_2H_4$ incorporated and converted to $^{14}CO_2$ by the pea seedlings was relatively small, amounting to 1.1 and 0.6 d.p.m. per mg dry weight per 20 h, respectively. During day 2, however, tissue incorporation and $^{14}CO_2$ release rapidly increased reaching a peak on day 3 of 12 and 33 d.p.m. per mg dry weight per 24 h, respectively, and then gradually declining during days 4 and 5. A peak of natural ethylene production preceded the peak in ethylene metabolism by approximately 24 h (Fig. 2*b*) and like ethylene metabolism gradually declined thereafter. In contrast to ethylene production and metabolism, respiratory CO_2 production remained relatively constant after reaching a peak of 17 μl per mg dry weight per 24 h on day 4 (Fig. 2*c*).

Considering the rigorous purification procedures used, it was highly unlikely that an impurity in the labelled ethylene may have still accounted for these results. Nevertheless, additional evidence was sought to further eliminate this possibility. The purified material was first pretreated with NaOH to remove possible acidic impurities or with $Hg(ClO_4)_2$, $KMnO_4$ or Br_2–H_2O to remove ethylene before exposure to pea seedlings. The results are presented in Fig. 3 and indicate that the incorporated radioactivity is derived from $^{14}C_2H_4$. Treatment with base had no effect whereas treatment with all three ethylene trapping agents eliminated the observed effect. Furthermore, regeneration of the $^{14}C_2H_4$ from the specific ethylene complexing reagent, $Hg(ClO_4)_2$ (ref. 13) completely restored activity.

Ethylene metabolism in these seedlings is almost certainly biological since essentially no radioactivity was recovered in the tissue or as $^{14}CO_2$ when the seedlings were heated to 80 °C for 1 min before $^{14}C_2H_4$ exposure or when the $^{14}C_2H_4$ was applied in anaerobic conditions. Additional evidence supporting this view, as well as indicating the complexity of the ethylene

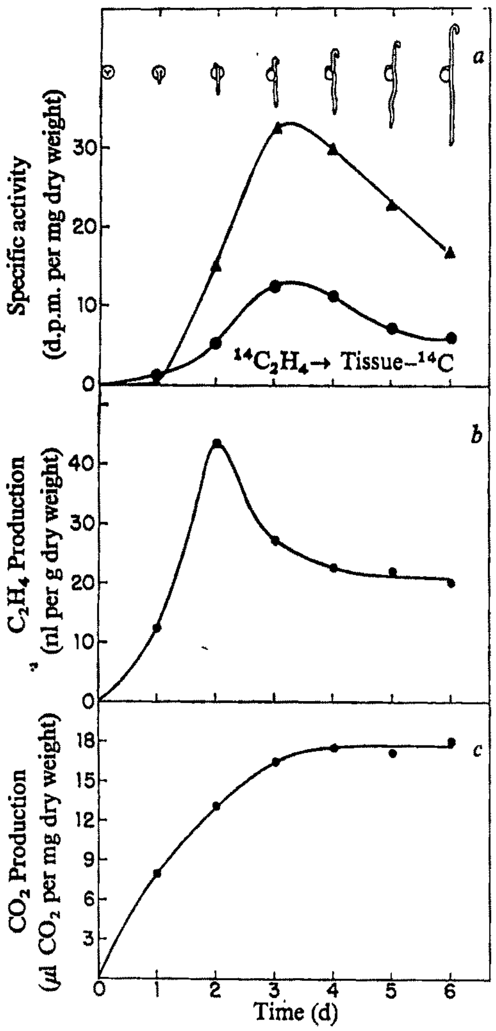

Fig. 2 *a*, Time course of $^{14}C_2H_4$ conversion to $^{14}CO_2$ (▲) and $^{14}C_2H_4$ incorporation into tissue components (●) of aseptically grown pea seedlings; *b*, natural ethylene production; *c*, natural CO_2 production. $^{14}C_2H_4$ specific activity was 22 mCi mmol^{-1}. Each datum point is the average of nine seedlings. Three such experiments were conducted with similar results. Pea seeds (*Pisum sativum* L. cv. Alaska) were rinsed with water and ethanol and surface-sterilised for 15 min in 1% sodium hypochlorite. The seeds were rinsed with 4 l of sterile water, treated for 10 min with 0.01 N HCl (ref. 14), rinsed again and placed in the dark in an equal volume of sterile water for 4 h at 27 °C. Following imbibition the seed coats were removed aseptically and the seeds rinsed four times with 4 l of sterile water. Sterilised seeds were germinated at 27 °C in the dark in Pyrex dishes between moist sheets of filter paper. Seedlings were transferred aseptically into 62 cm^3 flasks with glass centre wells and ground-glass stoppers. The freshly purified $^{14}C_2H_4$ was injected through a side-arm fitted with a 5 mm rubber serum stopper and the arm was immediately sealed off from the rest of the flask by rotating the stopper, as volatile substances from the serum stoppers often caused erratic results. Natural C_2H_4 and CO_2 production were determined in the same conditions as in (*a*) except that no $^{14}C_2H_4$ was added. CO_2 production rates were measured by liberating the CO_2 from the NaOH with acid followed by gas chromatography to determine the amount released. CO_2 production rates in conditions in which 10 µl l^{-1} ethylene was added were only slightly higher than in air. The radioactivity recovered in the NaOH was identified as $^{14}CO_2$ by liberating the CO_2 from the NaOH with acid and then isolating the CO_2 fraction by GC and comparing the radioactivity present in this fraction with the amount originally present in the NaOH. s.d. for $^{14}C_2H_4$ to $^{14}CO_2$ conversion (▲) in (*a*) for days 1 to 6 were ±0.7, 2.6, 5.8, 5.2, 1.4 and 0.4, respectively. The s.d. for $^{14}C_2H_4$ incorporation into the tissue (●) in (*a*) were ±0.2, 1.1, 2.4, 0.8, 0.3 and 0.2, respectively.

metabolism system, is the fact that ethylene metabolism was reduced over 80% if the cotyledons were detached from the seedlings during incubation of the whole seedling.

These experiments demonstrate that ethylene is incorporated and metabolised by intact pea seedlings. As yet, the possible physiological significance of this observation in terms of ethylene action is not clear. It is of considerable interest, however, that a peak of natural ethylene production should precede the peak in ethylene metabolism. This sequence does not seem to be the result of a delay in the peak of ethylene metabolism caused by a reduction in growth since the same sequence was observed in other experiments (E.B., unpublished) involving much shorter exposure periods in which the added ethylene had no detectable effect on growth. It is possible that ethylene action depends on its permanent incorporation into cellular components. Perhaps the peak in natural ethylene production and the peak in ethylene incorporation and metabolism reflects the progress of senescence in the cotyledons. The level of incorporation observed in these experiments could have been observed only through the use of $^{14}C_2H_4$ of high specific activity. I have found that CO_2, which often acts antagonistically to ethylene (ref. 1), will severely inhibit conversion of C_2H_4 to CO_2. This finding further suggests that ethylene metabolism and action may be closely linked.

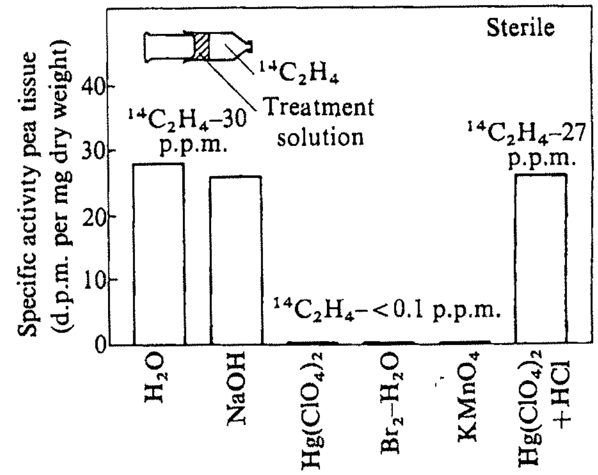

Fig. 3 The effect of pretreating purified $^{14}C_2H_4$ (specific activity 22 mCi mmol^{-1}) with water, NaOH, $Hg(ClO_4)_2$, Br_2–H_2O or $KMnO_4$ on its subsequent incorporation into aseptically cultured 2 d old pea seedlings. $^{14}C_2H_4$ (3 ml, 900 µl l^{-1}) was pretreated for 30 min over 1 ml of each treatment solution in a 5 ml glass syringe. The gas phase (2 ml) was then injected into a flask containing three pea seedlings and the seedlings were incubated for 24 h at 27 °C. Each datum point is the average incorporation of nine seedlings. The $Hg(ClO_4)_2$ solution was one-fifth the concentration recommended in ref. 13, thus eliminating the formation of impurities[8].

In view of the relatively large amounts of impurities found in all of the $^{14}C_2H_4$ samples purchased, it is difficult to assess the significance of previous studies. The instability of labelled ethylene is illustrated by the fact that within one week of purification, labelled radiolysis products could be detected. Previous studies[5,9,10] are further complicated by the fact that $^{14}C_2H_4$ was routinely regenerated from $Hg(ClO_4)_2$ before exposure to plant tissues. As reported by Jansen[8] and confirmed in similar experiments, this procedure leads to the formation of impurities which are readily incorporated into plant tissues. These criticisms raise considerable doubt as to the validity of previous studies and their significance must be evaluated on the basis of more critical experiments.

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Received December 9, 1974, accepted March 27, 1975

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Alcohol selection by DBA and C57BL mice arising from ova transfers

THE notion of the hereditability of alcoholism has alternated between acceptance and rejection[1–4]. Convincing evidence for a genetic involvement in man has been reported[5,6], but the issue remains controversial[7]. Because nature and nurture are so intertwined, and the methodology of their separation in humans is difficult, and even often suspect, inbred mouse strains have been used to examine the genetic basis of the disparate voluntary alcohol intakes seen in these strains[8,9].

The transfer of fertilised ova between strains of mice exhibiting extremes of alcohol choice separates the contributions of heredity and environment. Measurement of the retention, or the loss, of strain-typical preference or aversion for alcohol in the offspring arising from fertilised ova transferred to a mother of a different strain then enables an estimate to be made of the relative contributions of heredity and environment to alcohol selection. Our findings uphold the hereditary basis of alcohol selection in C57BL mice, but show the avoidance of alcohol drinking by DBA mice to be amenable to environmental alteration.

Ova donors and recipients were primiparous adult female C57BL/6 (alcohol preferrers) and DBA/2 (alcohol avoiders) mice born in our colony from breeder stock purchased from Jackson Laboratories. The mice were housed in plastic cages (19 × 29 × 14 cm) covered with a wire-mesh top indented to hold Purina lab chow and cylinders of fluid graduated to 0.2 ml. A cycle of 12-h light and 12-h dark was used. Fifty-four offspring were used as subjects; their grouping and sexual composition are shown in Table 1.

Ova were transferred by a modification of the method of McLaren and Michie[10]. Donor females were used 3.5 d and recipient females 2.5 d after identification of a vaginal plug.

At birth, the litters were culled to a maximum of four pups, since no more than three transfers were born to one female; the remaining pups, ranging from one to three, were her own. Differences in eye pigment identified experimental pups at birth. In the case of control transfers (C57BL to C57BL and DBA to DBA), litters were culled to a maximum of four without respect to sex. The untreated controls were mated and housed in the same manner as the experimental and control transfer animals, but subjected neither to anaesthesia nor to surgery.

Pups were weaned at 25 d and housed individually for 7 d; during this time they were offered a choice between tap water and 7.5% ethanol (w/v, prepared from 95% ethanol). The fluids were presented in two 25-ml cylinders graduated to 0.2 ml and fitted with stoppers and ball-bearing spouts. Daily fluid consumption was measured and the tubes were alternated; the subjects were weighed every other day. A mean preference ratio (ml 7.5% w/v ethanol per ml of total fluid per d) and mean fluid intake (alcohol, water and total fluid per g body weight per d) were calculated for each subject based on the 7-d test period.

Table 1 Distribution of subjects among experimental groups

Group	Male	Female	Donor	Recipient	Condition
1	5	2	C57BL	DBA	Experimental
2	3	5	C57BL	C57BL	Control
3	6	4	—	—	C57BL untreated control
4	4	6	DBA	C57BL	Experimental
5	4	6	DBA	DBA	Control
6	7	2	—	—	DBA untreated control

Table 2 Mean body weight and mean fluid intake (±s.e.)

Strain	Group*	N	Body weight (g)	Water intake ($\mathrm{ml\ g^{-1}\ d^{-1}}$)	Alcohol intake ($\mathrm{ml\ g^{-1}\ d^{-1}}$)†	($\mathrm{g\ kg^{-1}\ d^{-1}}$)‡
C57BL	1	7	12.7±0.7	0.036±0.013	0.303±0.032	22.7±2.4
C57BL	2,3	18	12.0±0.3	0.090±0.015	0.174±0.013	13.1±1.0
DBA	4	10	11.6±0.4	0.189±0.040	0.113±0.029	8.5±2.2
DBA	5,6	19	10.9±0.4	0.267±0.021	0.056±0.009	4.2±0.7

*Refers to groups of Table 1.
†ml of 7.5% (w/v) ethanol per g per d
‡g of ethanol per kg per d.

Since no sex differences were noted in either strain, the sexes were pooled and treated as one; the two control groups for each strain (untreated mice and transfers to the same strain) were not statistically different from each other and these data were also combined for analysis. The group means (± s.e.) for the various measures are shown in Table 2.

An analysis of variance for a complete orthogonal set of contrasts for the transfer and strain variables was carried out. Strain differences were highly significant for body weight, water and alcohol intake, preference ratio, and g ethanol per kg per day ($F = 5.73–65.2$, d.f. $= 1/50$, $P = < 0.000001–0.021$). C57BL mice weighed more, drank more alcohol and less water than the DBA mice. Collapsing across strains, treatment condition (transfer compared with non-transfer) was significant for water and alcohol consumption, preference ratio and g ethanol per kg per day ($F = 7.04–22.59$, d.f. $= 1/50$, $P = 0.000017–0.011$). Control mice of both strains drank less alcohol and more water than transferred mice. The strain × treatment interaction was significant only for the water, alcohol and g of ethanol per kg per day measures ($F = 4.29–10.26$, d.f. $= 1/50$, $P = 0.0024–0.044$). Mice arising from transferred ova of both strains thus drank less water and more alcohol than their respective control groups. No differences in total fluid intake were noted.

Our results support previous findings[8,9] that alcohol preference in the C57BL strain has a genetic basis, but provide even more conclusive evidence. Offspring arising from drinker ova transferred to the uterus of a non-drinker mother and raised by her not only retained, but increased their strain-typical high alcohol preference. We cannot offer a convincing (factually based) rationale for this increase. The offspring arising from fertilised non-drinker DBA ova transferred to drinker C57BL mothers also increased their alcohol intake and thus failed to retain their strain-typical aversion to alcohol, suggesting that either the prenatal or postnatal environment, or a combination of the two, is sufficient to increase alcohol consumption in DBA mice. Support for a positive postnatal rearing effect has been reported by Komura *et al.*[11] in normal (non-transferred) mice and we have confirmed[12] this; DBA offspring fostered by C57BL mothers from birth to weaning increased their voluntary alcohol consumption, but in C57BL weanlings (pups fostered by DBA mothers) alcohol intake was unaffected. These results do not, of course, preclude the possibility that the prenatal environment is of equal importance: a cross-fostering study of offspring from transferred ova would be required to partition accurately the maternal effects.

Our results support the genetic basis of alcohol selection in the C57BL mouse, the nature of which is still unknown, while demonstrating that alcohol avoidance in DBA mice is subject to environmental manipulation. The identification of those physiological and/or biochemical factors under genetic control and determining alcohol preference should appear high on any list for investigation.

Although research on the genetic aspects of alcohol preference in mice may serve to further the understanding of the predisposing role of inherited factors, any results garnered from such research cannot easily be generalised for their applicability to the problems of alcoholism in man.

We thank Dr Benson Ginsburg and Ms Alice Trottner (University of Connecticut) for help with the surgical procedure. This research was supported by grants from the Busch Memorial Fund (predoctoral fellowship to C.L.R.) and a biomedical sciences support grant from Rutgers University, the US National Institute on Alcohol Abuse and Alcoholism and the Distilled Spirits Council of the United States. C.L.R. is an NIAAA Postdoctoral fellow.

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Dominance at *Adh* locus in response of adult *Drosophila melanogaster* to environmental alcohol

IT has been argued[1] that natural selection acting on enzyme polymorphisms may be most clearly demonstrated by challenging polymorphic populations with an environmental additive acting as a specific substrate for the enzyme under study. Wills and Nichols[2], for example, showed that, in their experimental conditions, heterozygote advantage at the octanol dehydrogenase locus in *D. pseudoobscura* depended on the presence of octanol in the food medium. Similarly, de Jong *et al.*[3] found that the amylase^{4,6} variant increased strikingly in frequency in populations of *D. melanogaster* moved from a sucrose to a starch-rich food medium. $Amy^{4,6}$ is known to possess the highest *in vitro* activity on starch substrates of all common amylase variants[4]. We have examined the relationship between mortality and genotype at the alcohol dehydrogenase (*Adh*) locus when adult *Drosophila* are exposed to environmental ethanol.

Table 1 *Adh* genotypes among wild-caught *D. melanogaster* from a wine cellar and neighbouring rubbish tip

	Adh genotype			Frequency of Adh^F
·	Adh^F/Adh^F	Adh^F/Adh^S	Adh^S/Adh^S	± s.e.
Wine cellar	177	15	0	0.961±0.010
Rubbish tip	152	37	3	0.888±0.016*

*The two samples differ significantly $P<0.001$.

Wine cellars in Spain support extremely large populations of *D. melanogaster* that feed on, and breed in, the fermenting and maturing liquor (12–15% ethanol) impregnating floating mats at the surface of wine jars. Neighbouring rubbish tips and grape-skin composts also sustain populations. It seems probable that all the *D. melanogaster* within a town, and perhaps within a much larger region, form a single breeding unit, as we have observed considerable migration between bodegas (wine cellars). Recent studies of lethal allelism have indicated that samples of *D. melanogaster* collected at sites as much as 1.4 miles apart may belong to the same population[5]. We would therefore attribute any local differentiation of allele frequencies to selective values differing between sites, rather than to the sites representing independent discrete populations.

As might be expected, the Adh^F allele, whose enzyme product has a higher ethanol catalytic activity per individual than that of the Adh^S alternative, is at a high frequency (0.94–1.00) in cellar populations throughout Spain (D.A.B., J-M.M., and A.R., unpublished). The Adh^S allele is more favoured in non-cellar environments, being at a significantly higher frequency in a rubbish-tip sample than in our standard (Mancha) wine cellar, although the two sites are less than 1 km apart (Table 1). Similarly, the frequency of Adh^S increases dramatically in laboratory populations bred from large ($\sim 10^4$ adults) samples removed from wine cellars (D.A.B., J-M.M., and A.R., unpublished).

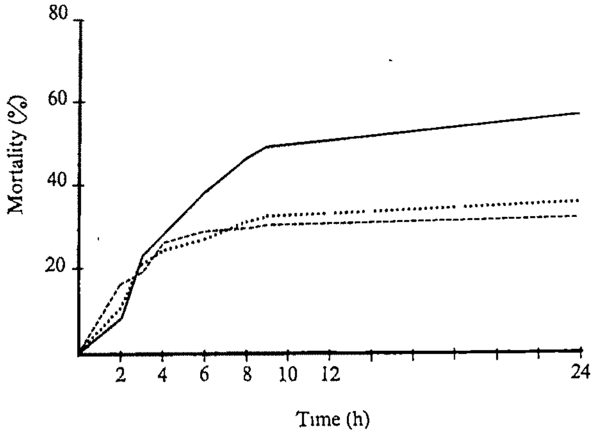

Fig. 1 Mortality/time plots for the three *Adh* genotypes when adult *D. melanogaster* are allowed to feed on medium containing ethanol (12.5%). ———, Adh^S/Adh^S; ...., Adh^F/Adh^S; ----, Adh^F/Adh^F.

We have tested the proposition that alcohol is the major environmental factor maintaining a high frequency of Adh^F in wine cellars by exposing adult flies to ethanol in a situation which mimics that confronting *Drosophila* entering a cellar and feeding on wine. Flies from the Mancha and Mandila population cages (which had been initiated 18 and 6 months previously with samples from two cellars 200 km apart), and from the standard Kaduna laboratory population, were placed in beakers. A Petri dish containing food (Edinburgh medium[6] supplemented with ethanol to a concentration of 12.5%) was fixed to the mouth of each beaker. The food was replaced every 2–3 h to maintain the concentration of ethanol. The *Drosophila* readily fed on this medium. Flies which died were removed and immediately electrophoresed to determine their *Adh* genotype. All individuals which survived were removed at 24 h and electrophoresed. No death occurred in controls in which the medium was supplemented with distilled water.

A typical mortality–time profile is shown in Fig. 1. Mortality is expressed as a percentage [(number of individuals of a given genotype dead after a given time/total number of that

Table 2 Mortality (%) of the three *Adh* genotypes after 24 h exposure to ethanol food

Population	*Adh* genotype Adh^F/Adh^F	Adh^F/Adh^S	Adh^S/Adh^S	Increase in frequency of Adh^F allele
Mandila (starved)	33.2 (211)	34.5 (258)	54.8 (62)	+0.0269
Mancha (starved)	43.2 (229)	45.6 (237)	74.5 (51)	+0.0430
Mancha (prefed)	12.4 (145)	16.5 (133)	36.4 (22)	+0.0192
Kaduna (starved)	68.2 (110)	69.9 (103)	91.7 (24)	+0.0612
Kaduna (prefed)	25.3 (99)	32.1 (109)	45.8 (24)	+0.0278

Total numbers of each genotype in the sample are given in parentheses.

genotype in the sample) × 100]. It is clear that mortality occurred predominantly during the first few hours of exposure. Accordingly the results for all samples have been expressed as total mortality (%) for each genotype after 24 h on ethanol food (Table 2). These data demonstrate a clear differential mortality between the genotypes, the Adh^S/Adh^S homozygote being significantly less resistant to alcohol poisoning than the heterozygote or Adh^F/Adh^F homozygote. The resulting increases in the frequency of Adh^F (frequency in survivors minus frequency in the sample before treatment) are given in Table 2. Increasing the rate of feeding, and hence of alcohol uptake, by starving the flies for 24 h in a humid chamber before treatment, significantly elevated the overall mortality.

In view of the strong selection against Adh^S in the presence of ethanol it is surprising that this allele is present at all in wine cellars. Presumably some form of balancing selection acting at other stages of the life cycle, or gene exchange with non-cellar populations, maintains the low frequency. Note that *Adh* alleles are not in linkage equilibrium with respect to other loci in Spanish populations, Adh^S being held in an inversion together with α-$Gpdh^F$ (D.A.B, J-M.M., and A.R., unpublished). Selection for or against Adh^S therefore acts on the entire contents of the inversion, and conversely, selection at other loci within the inversion will influence the frequency of Adh^S. Nonetheless, we consider that the observed mortality differences between genotypes are due primarily to the *Adh* locus, and not to selection acting on a linked locus, as the results from the Spanish populations are strictly comparable with those from the Kaduna population, which lacks inversions and detectable linkage disequilibrium.

McKenzie and Parsons[7] report that Chew failed to detect significant gene frequency differences at the *Adh* locus between Australian wine cellars and neighbouring non-cellar sites, and discount the alcohol dehydrogenase (ADH) system as a component of alcohol tolerance in *D. melanogaster*. Our results lead us to an opposite conclusion. If the treatment described above is a reasonable reflection of a natural situation we must conclude that the Adh phenotype expressed in an individual fly is an important component of its ability to tolerate environmental alcohol and, likewise, that adult mortality in the presence of ethanol-rich food plays a major role in maintaining a predominance of the high-activity Adh^F allele in wine-cellar populations.

Table 2 also shows that, in terms of survival on ethanol, the Adh^F allele is effectively dominant over the Adh^S alternative. This is at first sight surprising as all published data, and our own unpublished work, are in agreement that the *in vitro* ADH activity of *Adh* heterozygotes does not deviate consistently from the mid-point between the two homozygotes[8–11]. For example, the ratios of the mean specific activities of the Adh^F homozygote, heterozygote and Adh^S homozygote in the Mancha and Kaduna populations are 3.25:2.24:1.00 and 1.91:1.41:1.00, respectively (D.A.B, J-M.M., and A.R., unpublished). Additivity at the primary gene-product level but dominance at the physiological level are, however, the general observations, where data on inborn errors of metabolism are available[12,13]. Further, this relationship has been shown to be a general expectation derived from theoretical analyses of enzyme systems (ref. 14 and J. A. Burns, and H. Kacser, unpublished) and the data presented here support that prediction.

Dominance may influence the maintenance of a polymorphism in two ways. First, directional selection in a single environment becomes increasingly ineffective as the recessive allele becomes rare, thus dominance protects the recessive allele from extinction when a population encounters an environment in which the recessive is at a disadvantage. Secondly, should the dominance relationships be reversible under alternative conditions within a temporally or spatially varying environment, a marginal heterozygote advantage may result which could actively maintain the polymorphism.

We thank Norma Alexander for assistance. This work was supported by the ARC and the Juan March Foundation.

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Received February 4, 1975.

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Maintenance of allozyme polymorphisms in experimental populations of *Drosophila*

A CONTROVERSIAL point in modern population genetics is whether allozyme variation is adaptive or neutral. The interpretation of gene frequency distribution in terms of components of the environment[1–4] for a given species, or in terms of the comparison of patterns of allelic variation among different species[5,6], are cited as evidence of selection. Support for the alternative hypothesis derives from the apparent agreement between models based on neutrality and empirical findings either for the distribution of amino acid substitutions in proteins[7,8] or for the distribution of heterozygosity[9]. These arguments have been challenged on theoretical grounds[10,11], as well as on the basis of the distributions of allelic frequencies found in natural populations[12,13].

The selective value of allozyme polymorphisms may be assessed by testing allozyme systems under experimental conditions. We have used this method to study several experimental populations and look for changes in allozyme frequencies through the generations. Results reported here indicate that selection is probably responsible for the changes in gene frequencies observed in our experimental populations. These changes are, however, also influenced by the adaptive value of the genetic background that interacts with the allozyme genotypes under study.

The loci under study were *Est-5* (*X chromosome*), *Mdh-2* (IV) and *Odh* (II) in *Drosophila pseudoobscura*. For the *Est*-5 locus, 19 independent homozygous strains were obtained by brother×sister matings from the progenies of wild females collected recently at McDonald Ranch (Coast Ranges of Northern California). For the *Mdh-2* and *Odh* loci we similarly obtained 31 and 19 independent strains, respectively. This represents a large number of independent chromosomes sufficient to avoid any kind of large artificial linkage disequilibrium at the beginning of the experiment.

For each of these three enzyme loci two population cages (A and B) were started each with about 3,000 individuals. The founder individuals were obtained from the strains mentioned above, combined in appropriate numbers to start one cage (A) with a high initial frequency (0.80) of the rare naturally occuring allele (respectively, *104, 112* and *104* for *Est-5, Mdh-2,* and *Odh*), and another cage (B) with a low initial frequency (0.20) of that allele. The experimental populations were maintained and sampled at regular intervals for more than 600 d. Figure 1*a–c* shows the results. Gene frequencies have changed significantly in every one of the A cages, but the B cages do not show evidence of significant changes. The two *Est*-5 cages may have attained a common equilibrium level, but this result must await future generations of sampling for confirmation. Most significant is that all the changes in the A cages are in the same direction, namely towards the natural gene frequencies.

In every one of the A cages there was a large initial drop in the frequency of the rare allele. As a result of the rarity of this allele in nature, the original homozygous strains for this rare allele were more inbred than the homozygous strains for the commonest allele, and this may be the cause of the low initial fitness values of the genotypes with the rare allele. To test this possibility we crossed the original homozygous strains for the *Est*-5^{104} in pairs, using each strain in two crosses, as the male parental in one cross and as the female parental in a different cross. The F_1 progenies of these crosses were again crossed in pairs and this procedure was continued for five generations, to obtain a unique strain still homozygous for *Est*-5^{104} but with a largely heterozygous genetic background. This same procedure of matings was used with the strains homozygous for *Est*-5^{100}. With these two outcrossed strains we started two new population cages with high (cage 80) and low (cage 20) allele frequencies, similar to the A and B cages described above. Figure 1*d* shows the changes in gene frequencies observed during the first 500 d of sampling. The initial drop for allele *Est*-5^{104} of cage 80 is similar to that of cage A (Fig 1*a*); this rules out the effect of inbreeding as a major factor responsible for the initial drop of the rare allele in the original cages.

An interesting feature of the results (Fig. 1*d*) is that the *Est*-5^{104} allele now seem to be levelling off at a higher frequency than it did previously in cage A. *Est*-5 strains had been kept in the laboratory for one year (about 12 generations) before the serial crosses were started to obtain the outcrossed lines. Under the prolonged overcrowded environment of the cultures one might expect selection to favour genes which increase genotype efficiency to compete and to exploit this kind of environment (K-selection). Adaptive changes caused by modifier genes have been reported not only in allozymes[15,16] but also in morphological mutants[17–20] caused by directed selection or in response to laboratory conditions. We propose that selection for these modifier genes associated with the *Est*-5 locus may have occurred during this time, and that this may be a major reason for the increase in fitness shown by the genotypes with *Est*-5^{104} allele.

Yamazaki[22] did not find significant selective differences between two alleles of *Est*-5 locus in *D. pseudoobscura*. The flies used in his experiment, however, were extracted from an experimental population cage which had been previously kept in laboratory conditions for many years. These

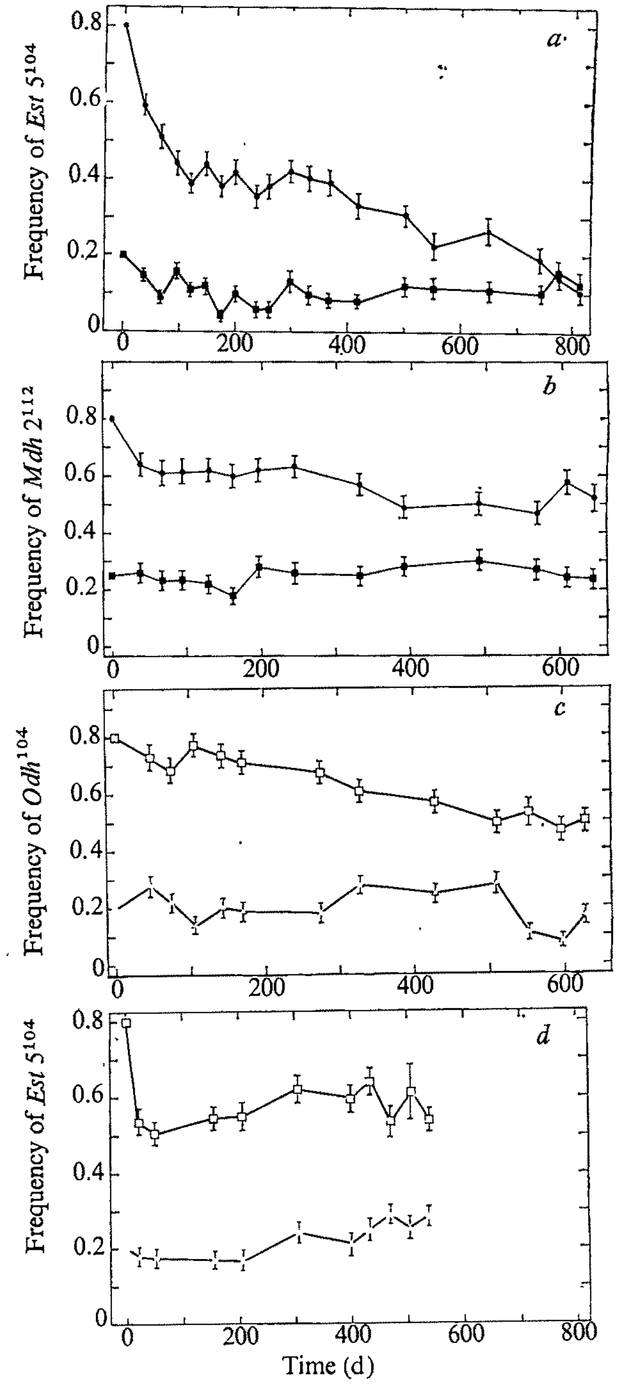

Fig. 1 *a*, Mean frequencies (95% confidence intervals) of allele *Est*-5^{104} of *D. pseudoobscura* in two experimental population cages, A (●) and B (■). The other allele present in the cages is *Est*-5^{100}. Each point is based on two samples of about 150 eggs taken from each cage during 3 consecutive days. Each individual sample of eggs was placed in a separate culture from which we sampled at random 24 emerged females. The genotypes of these females (a total of 144 per cage per sample) were analysed for the *Est*-5 locus using starch gel electrophoresis[24]. *b*, Allele *Mdh*-2^{112} in cage A (●) and B (▲). The other allele present in the cages is *Mdh*-2^{100}. Sampling as in (*a*) except that the total number of flies subjected to electrophoresis was about 96 per sample. *c*, Allele *Odh*^{104} in cage A (□) and B (△). The other allele present in the cages is *Odh*^{100}. Sampling as in (*b*). *d*, Allele *Est*-5^{104} in two experimental cages, 80 (□) and 20 (△). Sampling as in (*a*).

populations may well have built up a high adaptation to the experimental conditions through the selection of highly adapted favourable gene modifiers. The presence of these modifiers may obscure the detection of small differences in fitness between *Est*-5 alleles.

The fact that selection is not operating exclusively and directly on allozymes but also simultaneously on other interacting genes does not rule out selection as responsible for the maintenance of allozyme polymorphisms. The uniformity of response in all the initial cages (A, B) in the present experiment towards frequencies close to the naturally occurring ones, is not what would be expected if the allozymes were neutral and associated randomly with other genes subjected to selection. If this were the case, one should find a variety of responses[21].

Marinkovic and Ayala[23] have tested several fitness components for the same genotypes we used and their results are consistent with the view that selection is operating on the three loci we studied. Our results also indicate that selection must be acting on these loci, if not directly, at least through a series of coadapted gene complexes in which the allozymes genes are integrated.

We thank Miss Lorraine Barr for assistance. This work was supported by a US Public Health Service international fellowship, by a contract from the US Atomic Energy Commission and by a fellowship from the Ministry of Education, Spain.

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Do blue-eyed people have more sensitive corneas than brown-eyed people?

IT has sometimes been noted by opticians involved in the fitting of contact lenses that the sensitivity of the cornea seems to vary, depending on whether the patient has blue or brown eyes. Since it is likely that the sensitivity of the cornea reflects general tactile sensitivity, this knowledge could be valuable. Because there does not seem to be any quantitative report of this phenomenon, I carried out an experiment in which corneal sensitivity to touch could be compared among people with different eye colour.

The Cochet-Bonnet aesthesiometer[1] was used to stimulate the cornea. Two models of the instrument, with nylon monofilament of 0.12 and 0.08 mm diameter, were used to produce pressures from 11–200 mg/0.0113 mm^2 and from 2–90 mg/0.005 mm^2, respectively. Both aesthesiometers are interchangeable and had previously been calibrated. The aesthesiometer was mounted in a holder such that it could be moved in *x*, *y* and *z* axes by means of three knobs. Thus, it was possible to achieve reliability in stimulation of a corneal point, a steady speed of application and a perpendicular corneal contact. A corneal point near the limbus in the six o'clock position was stimulated and the slightest bend of the nylon wire visible through a magnifier was defined as corneal contact. The inferior corneal point was chosen because the touch threshold measured there is not affected by psychological factors[2].

Measurements of corneal touch thresholds were made subjectively[3]. The experiment began with stimulation of the cornea with the lowest pressure and continued in an ascending fashion. At each predetermined length of the nylon monofilament (with increments equal to 0.5 cm with each instrument), four to six contacts were made with at least one blank to test the subject's reliability. The subject indicated when he felt the probe by pressing a bell. From these measurements the corneal touch threshold was defined as the length of the monofilament at which the subject responded for 50% of the number of stimulations. This length was converted into pressure using a previously calibrated curve for the instrument. The tolerances for this test are within ±5% as evinced by repeated measurements on the same subjects.

Caucasian subjects (112) 21–37 yr old (mean 23.5) participated in this experiment. Another group of 44 non-white subjects 22–33 yr old (mean 24.2) comprising 12 Negroes, 15 Indians and 17 Chinese, also served as subjects. As all these people (except four) were university students, they were about the same age in each group. All subjects were free of ocular pathology. The

Fig. 1 Relative corneal sensitivity to touch as a function of the colour of the eye. The dark brown group consists of the non-white subjects. The sensitivity is the reciprocal of the touch threshold measured in mg mm^{-2} and the group with the greatest sensitivity has been assigned 100% in this diagram.

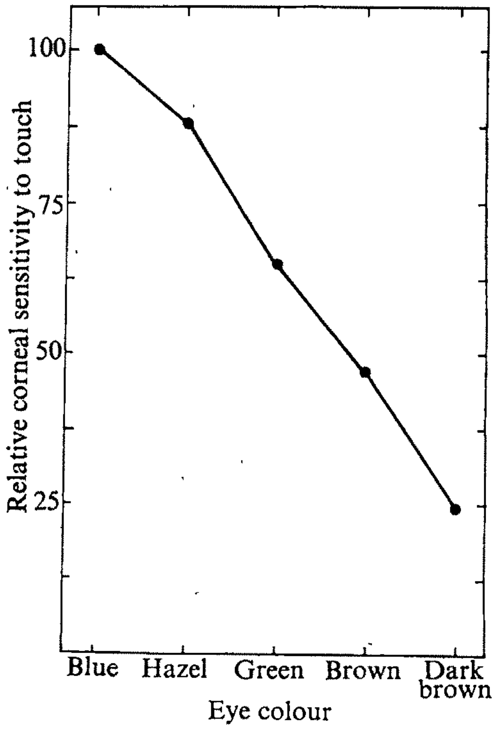

Table 1 Corneal touch threshold ($mg\ mm^{-2}$) in people with different iris colour

	Blue	Hazel	Green	Brown	Dark brown			Total
					Indians	Negroes	Chinese	dark brown
No. of subjects	39	12	12	49	15	12	17	44
Mean	14.3	16.2	22	30.4	47	61.6	64.5	57.8
S.d.	4.7	7.4	13.3	19.0	14.8	22.5	21.2	20.8

subjects were separated into groups according to the colour of the iris: blue, hazel, green, brown and the darker brown eyes of the group of non-whites.

The corneal touch thresholds obtained in variable order with these people are given in Table 1. These values were converted into sensitivity (threshold^{-1}) and the greatest sensitivity value was assigned 100%. The relative corneal sensitivity to touch for the various groups is illustrated in Fig. 1, in which there is only one group of people with dark brown eyes representing the non-white subjects.

The results of this study provide strong evidence that people with blue irises have the most sensitive corneas and people with brown eyes least sensitive; the sensitivity further diminishes in non-whites with darker pigmented eyes. It can also be seen (Fig. 1) that blue-eyed people have, on average, twice as sensitive corneas as white people with brown eyes (difference statistically significant, $P < 0.001$). The mean corneal touch threshold (and standard deviation) of all Caucasian females ($n = 55$) was 23.91 $mg\ mm^{-2}$ (s.d. 16.01) and of all Caucasian males ($n = 57$) 21.06 $mg\ mm^{-2}$ (s.d. 14.9). The difference between sex is not significant ($P > 0.5$), although it is worth noting that females are slightly less sensitive than males by about 10%. This factor may be accounted for by the fact that women have much less sensitive corneas for about 3 days at about the time of menstruation compared with the rest of the month[4].

It also seems from the present study that non-white people who have dark brown irises have much less sensitive corneas than white people. The difference between the group of white people with brown eyes and the non-white is significant ($P < 0.001$). The present results show that non-white people have, on average, four times less sensitive corneas than blue-eyed people and half as sensitive corneas as whites with brown eyes.

It could be argued that the variations displayed here were caused by the fact that corneal sensitivity varies diurnally[5] but this is unlikely as the diurnal variation is considerably smaller than the differences between eye colour.

This phenomenon is obviously relevant to the wearing of contact lenses. It may also have some bearing on problems of anaesthesia as it is possible that different quantities of anaesthetics may be needed according to eye colour. It may be inferred that corneal sensitivity reflects the general sensitivity of the body; and there is some evidence supporting this view as it was found that at about the time of menstruation corneal sensitivity is reduced[4], and the touch sensitivity of the middle finger is diminished[6].

This hitherto unreported phenomenon may help to explain large variations in corneal sensitivity in normal subjects. This variation can be puzzling when comparing the effect of some treatment or disease on the eye against a group of control subjects; this has already been noted in a study[7] of corneal sensitivity in diabetics.

It is already a known clinical fact that, in the eye, more drugs are needed on people with dark rather than with light-pigmented irises to obtain the same effect (for example cycloplegia). The reason for this is not known although it may be related to the amount of melanin pigment present in the iris, where ocular drugs have an effect. Since the cornea does not contain any such pigment, however, it is not easy to explain the diminution of corneal sensitivity with darker pigmentation of the iris. It is not known whether the thickness of the cornea varies with eye colour. It has been shown[8] that thicker corneas, as a result of oedema, are less sensitive than normal corneas. It is also unknown whether nerve density varies widely among these different people. An alternative possibility is that the difference in sensitivity may arise in the central nervous system and not at the periphery. The difference in tactile sensitivity may also be relevant to understanding that the practice of acupuncture may be more acceptable in China than it is in countries inhabited by blue-eyed people.

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Iris colour and relationship of tyrosinase activity to adrenergic innervation

IRIS colour to some degree depends on the sympathetic innervation of the eye. Chance clinical observations of humans and experimental studies in animals have demonstrated consistently a gradual lessening of colour intensity in the iris after interruption of the sympathetic pathways to the eye[1,2]. Similarly, full iris pigmentation fails to develop when the sympathetic innervation to the eye is absent from birth[3]. Interest in the relationship of sympathetic innervation to iris colour has increased with reports of direct adrenergic innervation to iris stromal melanocytes[4–6]. For mammals this is extraordinary in that most melanocytes, for example those of the skin, have no innervation. We have investigated this relationship using tyrosinase activity as an index of melanin synthesis since melanin is the chief pigment of the iris and tyrosinase is the rate-limiting enzyme in the production of melanin[7]. In turn, iris tyrosinase activity was measured in normal rabbits and in rabbits in which the sympathetic pathways to the eye have been interrupted.

Twenty-eight brown-eyed, Dutch belted rabbits of either sex with an average weight of 2.3 kg were used. In 10 animals the superior cervical ganglion on one side was resected under direct visualisation whereas in 18 animals the preganglionic nerve trunk was interrupted. To distinguish between the two types of operation the terms denervation and decentralisation are commonly used[8]. The occurrence of ptosis and miosis postoperatively was accepted as evidence that surgery had been successful. Survival times varied from 1 to 370 d. In all animals that survived longer than 2 months, the iris on the operated side became noticeably lighter in colour than that in the other eye.

Tyrosinase assays were performed in duplicate according to a modification of the method of Pomerantz[9]. Iris tissue, either homogenised or intact, was placed in a solution containing sodium phosphate buffer, 0.01 M, *p*H 6.8, and L-tyrosine-3,5-^{3}H, 10^{-5} M, specific activity 50 $Ci\ mol^{-1}$. The mixture was incubated at 37 °C for 2–5 h. At the end of the reaction period, the material was treated with Norit SG to remove all tritiated substrate as well as tritiated reaction

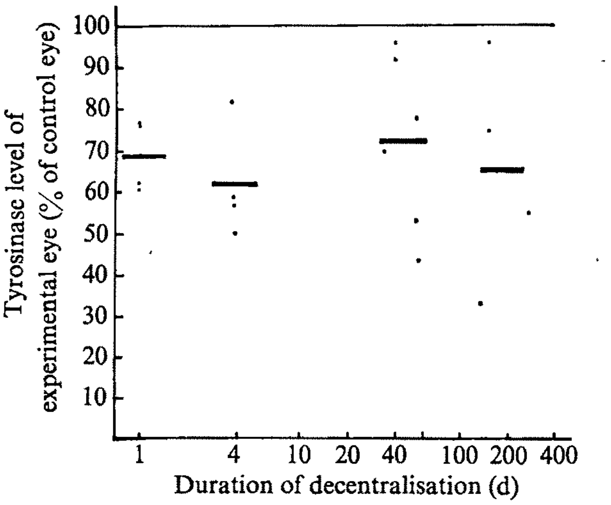

Fig. 1 After decentralisation of the superior cervical ganglion, iris tyrosinase activity declines rapidly. Once diminished, tyrosinase activity continues at the new, lower level for many months.

products except for tritiated water. An aliquot of the tritiated water was counted in a liquid scintillation counter. The activity of samples from iris tissue was calculated on the basis of c.p.m. per mg wet weight of tissue. Blanks for the assays consisted of either the reaction mixture without iris tissue or the reaction mixture plus iris tissue from an albino eye.

After either decentralisation or denervation, enzyme activity decreased in the ipsilateral eye. The results for both sets of animals are summarised in Table 1. The magnitude of the decline in iris tyrosinase was the same irrespective of surgical procedure. It should be remembered that the iris not only contains innervated iris stromal melanocytes but also has a posterior double lining of densely pigmented epithelial cells. Thus the proportion of the decline of iris tyrosinase activity that is attributable only to the iris stromal melanocytes cannot be told from these studies

When the assay results were plotted to show tyrosinase activity as a function of duration of decentralisation (denervation is similar but not illustrated here) (Fig. 1) we found that enzyme activity in the iris decreased within 1 d and remained depressed for at least 200 d Further, the assays of tyrosinase activity in the choroid (the posterior part of the uvea) in the same eyes yielded a similar rapid and long lasting decrease in enzyme activity.

In the face of such a clear-cut result, it seems reasonable to suggest a failure of maintenance, that is, to ascribe the lightening of iris colour that follows interruption of the sympathetic pathways to the eye to the inadequate replenishment of melanin within iris stromal melanocytes Thus as the melanin of the iris is slowly depleted by normal metabolic processes, the iris gradually loses colour. Although there is no electron microscopic evidence at present for metabolism of iris melanin[10], there has been a gradual build-up of information concerning degradation of melanin-containing bodies (melanosomes) in epidermis[11] and in certain preparations *in vitro*[12].

In its dependency on adrenergic innervation, ocular tyrosinase is not unique; it has become clear that an intact innervational network is a prerequisite for the regulation of enzyme levels in several organs innervated by the sympathetic nervous system. Perhaps the most interesting example of this type of regulation occurs in the pineal gland where under the influence of noradrenaline, levels of *n*-acetyltransferase can increase thirtyfold in a few hours[13–14].

Table 1 Effects of operation on iris tyrosinase activity

		Operated side as % of control side
Denervation*	Mean	70.0
	s.e.	5.4
Decentralisation†	Mean	67.4
	s.e.	4.2

* $n = 10$.
† $n = 18$.

We thank John Hendee, Maria Smith and Barbara Briggs for technical assistance. This work was supported by grants from the US Public Health Service, Research to Prevent Blindness, Inc. (to A.M.L.) and the American Cancer Society (to A.B.L.).

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Received February 3; revised March 24, 1975.

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Adult thymectomy results in loss of T-dependent mitogen response in mouse spleen cells

THERE is overwhelming evidence that murine thymocytes represent a heterogeneous population of cells that can be divided into discrete subpopulations on the basis of surface antigen markers[1,2], drug sensitivity[3], physical characteristics[4,5] and responsiveness to mitogens[6]. There is some indication that a similar heterogeneity with regard to biological function exists within the peripheral thymus-derived (T) lymphocyte population (reviewed in refs 7–9). Several investigators have shown that thymectomy of young adult mice reveals a differential sensitivity of several T-cell activities to this surgical procedure and suggested the existence of T-cell subsets with different life spans[7–9]. Therefore we investigated the response to PHA and concanavalin A in adult thymectomised (ATX) mice to determine if responsiveness to these mitogens could be attributed similarly to subpopulations of T cells.

We report here that spleen cells from BDF$_1$ mice thymectomised when 4 weeks old exhibit an initial increase in T-dependent mitogen responsiveness over age and sex matched littermate sham-operated controls (STX) followed by a rapid and sustained decay of responsiveness to these same mitogens, and that the response of identical cell preparations to a thymus-independent B-cell mitogen, lipopolysaccharide (LPS), does not show this divergence of responsiveness when thymectomised animals are compared with controls.

Of five experiments carried out, all gave similar results, and Fig. 1 shows the results of a representative experiment.

Adult thymectomy initially caused an increase in the response of spleen cells to phytohaemagglutinin (PHA) and con A. This was soon followed by a precipitous decrease in the level of con A and PHA responsiveness of cells from ATX animals while those from STX mice continue to increase to their mature adult level of responsiveness.

The response to pokeweed mitogen (PWM) (not shown here) was also lower in ATX animals although the differences were not as striking. In contrast, the response to LPS, though erratic, was similar in both normal and ATX mice. The 50% depression in these animals 20–38 weeks after thymectomy was not a consistent finding; in some experiments ATX cells gave a higher response than STX cells in these older mice. Most investigators agree that this mitogenic response occurs in the absence of T cells. If any T-cell component is involved, it is too small to have been detected in these experiments.

We have explored the response of these animals further and examined the kinetics, mitogen dose–response, variation in the number of cells cultured, and the substitution of foetal bovine sera for adult human sera. In all cases the responses of ATX animals to PHA and con A were depressed between 8 and 26 weeks after thymectomy compared with STX controls.

The decay in mitogen responsiveness seen here is consistent with the existence of two populations of T cells—one required for the bulk of the response to mitogens which decays completely by 9 weeks after thymectomy, and another long lived, responding poorly to PHA and con A. Although there have been several reports that adult thymectomy has no effect[10], or a transient effect[11–13] on mitogen responsive cells, some investigators have observed a decline in certain T cells subsequent to the same procedure. Raff and Cantor[7] and Kappler *et al.*[8] have demonstrated a diminution of precursors of T helper cells within 6 weeks after adult thymectomy. Futhermore, the number of Thy 1.2 positive cells in the spleen decreases by 5 weeks after adult thymectomy, caused primarily by a loss of those cells with a high rather than a low density of that antigen[9]. Moreover, rapidly dividing T cells found primarily in the spleen of normal mice are more sensitive to the cytolytic activity of anti-Thy 1.2 (ref. 14) and are required for the mitogenic response to PHA[15]. These results have been attributed to the presence of a short lived splenic T cell responsible for PHA responsiveness and possessing a high density of Thy 1.2.

The time course of the response in ATX mice (Fig. 1) is not clearly separable into decay curves of two cell populations. It therefore could be argued that it represents the decay of one long lived cell activity. In this case mitogen responsiveness should not drop off so rapidly unless maintenance of existing T-cell activity is thymus-dependent. Removal of the thymus does, of course, prevent further maturation of cells in the thymus and periphery. To the extent that maturation results from peripheralisation of cells from the thymus, thymectomy would abolish emigration. A deficiency in continued maturation would suffice to explain the inability of ATX mice to achieve the high levels of PHA and con A responsiveness seen in controls between 10 and 20 weeks of age.

The lesion in mice thymectomised as young adults is seen here as an inability to reach mature levels of responsiveness to PHA and con A and a decline of existing reactivity to these mitogens by loss of either a responding cell or an

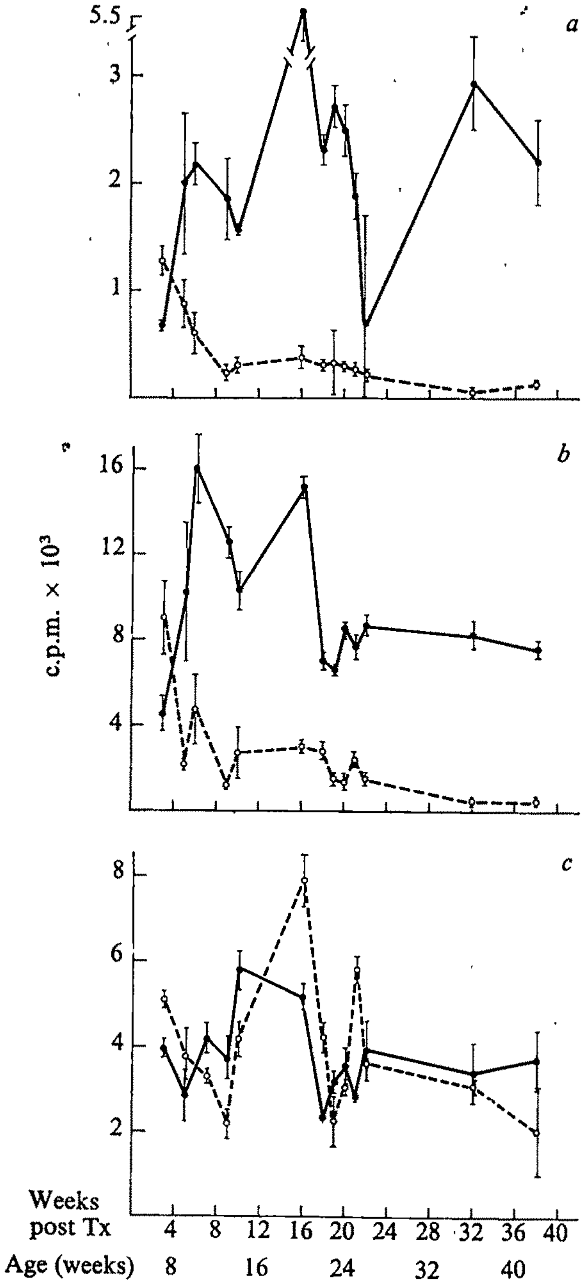

Fig. 1 Responses of 2.5×10⁵ spleen cells from ATX (○) and STX (●) BDF₁ mice to (*a*) PHA, (*b*) con A, and (*c*) LPS at indicated times after thymectomy. All 26 pools of spleen cells were cultured simultaneously with the various mitogens. Female BDF₁ (C57BL/6 female×DBA/2 male) mice bred at the University of California, San Diego, or purchased from Jackson Laboratories were used in all experiments. They were subjected to complete thymectomy (TX) or sham-thymectomy at 4 weeks of age, under light Avertin anaesthesia according to the technique of Dischelar and Rudali[16]. On the day of the experiment animals were killed by cervical separation, the spleens removed aseptically and single cell suspensions prepared as described previously[17]. Counts and viability determination were obtained using a haemocytometer and trypan blue dye exclusion. The cells were then resuspended in RMPI-1640 (GIBCO) which was supplemented with 10% adult human serum from one of us (W.B.). 2.5×10^5 cells in 100 µl were then added to individual wells in microtitre plates (Cooke Engineering Co., Alexandria, Virginia) and a further 100 µl added of either mitogen in serum-free medium or serum-free medium alone in the case of non-stimulated background cultures. Mitogens were used at the following final concentrations: 2.5 µg ml^{-1} PHA (Wellcome Research Laboratories, Beckenham, Kent), 10 µg ml^{-1} con A (Pharmacia), 20 µg ml^{-1} LPS (LPS W from *E. coli* 055:B5, Difco). The cultures were incubated at 37 °C for 44 h and then pulsed with 50 µl of ^{125}I-5-iodo-2-deoxyuridine (^{125}IUdR) (1 µCi ml^{-1}, specific activity >200 Ci mmol^{-1}, New England Nuclear Corp.) for a further 12 h. The cultures were then removed and collected using a slightly modified multiple cell culture harvester[18] (Otto Hiller Corp., Madison, Wisconsin). The cells collected on to the filter paper were rinsed with saline, 15% trichloroacetic acid and 95% ethanol before being counted in a Nuclear Chicago automated gamma-counter. Cells cultured without added mitogen contained less than 115 c.p.m. Data given are mean c.p.m. of four cultures ±s.d.

amplifying cell. Our data do not permit a precise evaluation of the half life of this activity.

We thank Ms Mary Coleman for technical assistance. This work was supported by grants from the National Institute for Allergy and Infectious Diseases, the National Cancer Institute, the National Foundation, the Rockefeller Foundation, and the Cancer Coordinating Committee of the University of California, and a fellowship from the Cancer Research Institute, Inc.

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Received December 23, 1974, revised February 27, 1975.

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Specific lymphocyte-activating determinants expressed on mouse macrophages

WHEN two populations of allogeneic lymphoid cells are cultured together, cellular interactions lead to blast transformation and proliferation of a portion of the cultured cells. In mice, this mixed leukocyte culture reaction (MLR) is under the independent control of two genetic systems: the multigenic major (H-2) histocompatibility system (MHS)[1,2] and the unigenic (non-H-2) *M* locus[3]. The nature and cellular distribution of the gene products which mediate recognition of one cell (the stimulator cell) by another cell (the responder cell) are incompletely defined. The 'recognising' structure is likely to be a specific receptor present on thymus-derived (T) lymphocytes, since T cells are generally required as responder cells in MLR[3-6]. Recently it has been possible to distinguish, from these recognition sites, gene products which are expressed on the stimulator cells[7] and which have been termed lymphocyte-activating determinants (LADs)[8]. Cell fractionation studies revealed that LADs of the MHS are expressed on both B and T lymphocytes[8,9], whereas LADs of the *M* locus are preferentially if not exclusively expressed on B lymphocytes[10]. In this study we present evidence that LADs of the *M* locus as well as LADs of the MHS are also expressed on macrophages. These genetic determinants are shown to be stable in long term cultures and to induce specific one-way reactions in mixed lymphocyte–macrophage cultures.

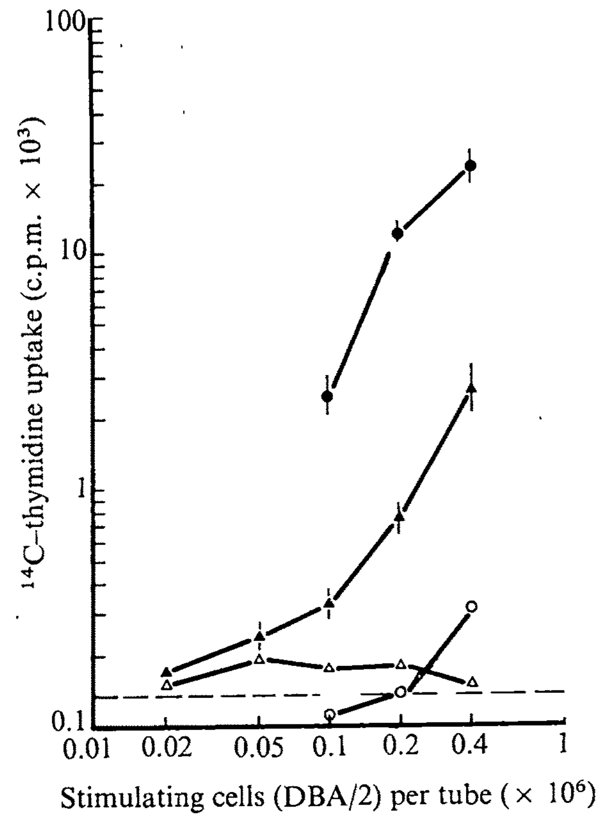

Fig. 1 Dose-response curves of mixed culture reactions with varying numbers of DBA/2 macrophages (●) or DBA/2 lymph node lymphocytes (▲) as stimulating cells and 0.6 × 10^6 BALB/c lymphocytes per tube as responding cells. Open symbols, respective stimulating cells alone. ^{14}C-thymidine uptake was measured on day 2–3. Dashed line indicates the uptake by the responding cells cultured alone.

Macrophage monolayers were prepared in flat-bottomed 9 × 44 mm polystyrene tubes using 4 × 10^5 macrophages obtained from the peritoneal cavity of normal mice (J. R.-M., V.S., F. Garrido and H.F., unpublished). After removal of non-adherent cells by several washings 6 × 10^5 lymphocytes obtained from lymph nodes of allogeneic mice were added in a total volume of 0.4 ml. These mixed cultures were incubated for 48 h at 37 °C in an atmosphere of 10% O_2, 4% CO_2 and 86% N_2. ^{14}C-thymidine (20 µl; 60 mCi mMol^{-1}) was then added and the tubes were incubated for a further 16 h. The non-adherent cells were collected on to a filter using a semi-automatic harvester and their isotope uptake measured in a liquid scintillation spectrometer.

Mouse lymphocytes cultured with allogeneic or semi-

Table 1 Proliferative response of mouse lymphocytes cultured with allogeneic or semiallogeneic macrophages

Incompatibility*	Stimulating macrophages	Responding lymphocytes	^{14}C-thymidine uptake†	Factor of stimulation‡
M locus	(BALB/c × DBA/2)F_1	BALB/c	10,307 ± 146	9.1
	BALB/c	(BALB/c × DBA/2)F_1	1,133 ± 125	1
H-2	(BALB/c × CBA/H)F_1	BALB/c	5,601 ± 416	5.2
	BALB/c	(BALB/c × CBA/H)F_1	1,086 ± 246	1
M locus	DBA/2	BALB/c	12,453 ± 435	12.7
	BALB/c	DBA/2	978 ± 12	1

* BALB/c ≐ H-2^d, Mls^b; DBA/2 = H-2^d, Mls^a; CBA/H = H-2^k, Mls^b.

† Mean c.p.m. ± s.d. from triplicates; uptake of the macrophages cultured alone was in the range 300–500 c.p.m.; uptake of the lymphocytes cultured alone was in the range 50–200 c.p.m. Uptake by F_1 lymphocytes cultured with parental macrophages or of DBA/2 lymphocytes cultured with BALB/c macrophages was not significantly higher than the uptake by syngeneic cultures (not shown).

‡ Comparing uptake obtained in one direction with the uptake obtained in the other direction.

Table 2 Proliferative response of mouse lymphocytes cultured with highly purified allogeneic or semiallogeneic macrophages

Stimulating macrophages*	Responding lymphocytes†				
	None	BALB/c	DBA/2	(BALB/c × DBA/2)F_1	AKR/J
—		65	84	41	84
BALB/c	77	762 (1)	393 (1)	338 (0.3)	1,237 (3§)
DBA/2	146	13,943 (18‡)	279 (1)	303 (0.3)	1,440 (3.5§)
(BALB/c × DBA/2)F_1	114	4,793 (6‡)	243 (1)	995 (1)	2,078 (5§)
AKR/J	90	7,786 (10‖)	1,099 (4§)	2,594 (3§)	404 (1)

* Peritoneal macrophages were precultured for 10 d. More than 95% of the monolayer cells stained for lysosomes with acridine orange. No lymphocytes or PMN could be detected The monolayers were washed, incubated with 0.25% trypsin solution for 30 min at 37 °C, washed again and then used as stimulating cells.

† ^{14}C-thymidine uptake in c.p.m. (mean of triplicates) obtained in the various cultures contained lymphocytes alone (first horizontal row). macrophages alone (first vertical row) or both together in all possible combinations. The numbers in brackets indicate the factor of stimulation based on the uptake observed in the syngeneic mixed cultures

‡ Incompatibility at the *M* locus (unilateral stimulation by LAD of Mls^a); AKR/J = H-2^k, Mls^a; for other strains see Table 1.

§ Incompatibility at H-2.

‖ Incompatibility at the *M* locus and at H-2.

allogeneic macrophages showed specific proliferative responses (Table 1). Parental lymphocytes responded well to F_1 macrophages differing either at the *M* locus or at the H-2 complex, but F_1 lymphocytes did not respond to parental macrophages. The one-way reaction in the lymphocyte–macrophage mixed cell culture system is further demonstrated by the results obtained with cells from the *H-2*^{d} identical mice DBA/2 and BALB/c. Unilateral stimulation of BALB/c by DBA/2 has been reported with lymphocytes as stimulator cells[11] and this has been attributed to the strong lymphocyte-activating determinant coded for by the *Mls*^{a} allele at the *M* locus[12]. It therefore seems that LADs of the H-2 complex as well as LADs of the *M* locus are expressed on macrophages.

The possibility that stimulation in our mixed cultures was caused by contaminating lymphocytes or polymorphonuclear cells (PMN) was excluded in various ways: (1) pretreatment of the macrophage monolayers with anti-B cell and anti-T cell serum[13] plus complement; (2) pretreatment of the monolayers with trypsin combined with extensive washing (J. P.-M., V.S., F. Garrido and H.F., unpublished); (3) preculturing the macrophages for about 10 d thus allowing PMN[14] and probably also lymphocytes to die; and (4) combining methods (2) and (3). None of these procedures reduced the stimulating capacity of the macrophage monolayers. Table 2 shows an experiment in which macrophages obtained from 10 d cultures were treated with trypsin to detach any remaining lymphocytes and to remove dead cell material, and extensively washed before they were used as stimulator cells in mixed cultures. Specific proliferative responses were again observed when the cells were incompatible at the *M* locus, at the H-2 or both.

Interestingly, *M*-locus incompatibility between lymphocytes and macrophages resulted in quite strong stimulation, whereas H-2 incompatibility only led to comparatively weak stimulation. Figure 1 shows a dose–response curve of the stimulation obtained in an *M* locus incompatible combination (DBA/2 against BALB/c). DBA/2 macrophages seemed to have stronger stimulatory capacity than DBA/2 lymphocytes. Further experiments are in progress to determine the relative stimulatory capacity of macrophages compared with other non-lymphoid or lymphoid subpopulations of cells, and to establish the genetic mapping of the LADs expressed on macrophages by testing congenic and H-2 recombinant mice.

Studies on macrophage–lymphocyte interactions in MLR date back to the early days of the MLR. Although there is general agreement that macrophages are required in the reaction, reports on their specific contribution to the allogeneic stimulus have been conflicting. Some of the results are difficult to interpret because the numbers of monocytes or macrophages were not quantitated. Wherever human purified macrophages were tested directly, they were found to be powerful stimulators of allogeneic lymphocytes[15,16]. Other non-lymphoid human cells known to carry histocompatibility antigens on their surface have usually not been found to stimulate allogeneic lymphocytes *in vitro*. In the guinea pig, macrophages were recently found to be the predominant stimulator of the MLR, far more effective than lymphocytes, fibroblasts or PMN[17]. Mouse peritoneal macrophages were found to be immunogenic not only in MLR, as described here, but also when used for induction of cytotoxic cells[18] or cells exhibiting the graft-versus-host reaction[19].

The importance of the macrophage for the immune system has recently been firmly established. Many observations suggest a function for the macrophage in antigen presentation. This may explain their requirement, for example, in soluble antigen-mediated T lymphocyte proliferation[20], MLR[21], induction of helper T lymphocytes[22] and cytotoxic effector cells[23], and in T–B cell cooperation *in vitro* leading to antibody synthesis[24]. Macrophages have also been found to be involved in the genetic control of certain immune responses[25]. Cooperation between macrophages and T cells was found to depend on a certain compatibility, which was associated with the I region, possibly with Ia antigens, of the H-2 complex (P. Erb, personal communication). Clearly, the study of macrophage cell-surface components, like Ia antigens and LADs will become increasingly important for the understanding of the nature of macrophage–lymphocyte interactions.

This work was supported in part by the Cancer Research Campaign and the Wellcome Trust. We thank Mrs Barbara Reynolds for assistance.

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Control of flagellar wave movement in *Crithidia oncopelti*

MECHANISMS controlling cell behaviour are of considerable importance in biology and medicine, and much effort has been expended in experiments designed to evaluate the biochemical events which are involved in the mediation of a variety of cellular activities. Calcium ions are found to be involved in the regulation of a number of motile processes, such as muscular contraction[1], ciliary reversal[2] and cytoplasmic contraction[3]. We present here preliminary results which show that calcium may be responsible for the control of flagellar activity in a trypanasomid flagellate, *Crithidia oncopelti*. The flagellum of this organism propagates waves both distally and proximally in common with other trypanosomes[4] but in contrast to the flagella on the majority of other protozoa and spermatozoa which are observed to maintain only distally propagating waves[5]. During normal swimming *Crithidia* propagates waves from tip to base, but on meeting an obstruction wave reversal occurs for several flagellar beats, thereby causing the organism to reverse its direction of movement and enabling it to escape from the obstacle. The wave reversal thus seems to be an avoidance reaction, and can be induced by mechanical, electrical or chemical stimulation[6].

Cells of *Crithidia* have been extracted in glycerol[7] and detergent-based[8] solutions in such a way that the addition of ATP and magnesium to the preparation induces wave motion. The character of the reactivated flagellar movements is critically dependent on a number of physical and chemical factors during extraction and reactivation; with the detergent-based technique higher flagellar beat frequencies are obtained than for corresponding conditions with the glycerol-based method. In a previous report on the behaviour of glycerol-extracted cells[7] the wave direction was from the basal end of the flagellum only, which seemed to have lost the ability to reverse the direction of wave propagation. In those experiments, however, no attempt was made to control the concentration of calcium ions, which we have now found to have a significant effect on the behaviour of the system.

In our experiments the reactivating solutions contained ATP and magnesium at a concentration of 1 mol m^{-3} (1 mM) and the concentration of free calcium in the solutions was controlled by the addition of EGTA. The amount of calcium in the solutions was determined using an atomic absorption spectrometer, and the concentration of free calcium ions was calculated using a method described previously[8]. Unless the calcium concentration was low in both the extraction and reactivation solutions, waves are propagated from the base of the flagellum only. When the concentration of calcium is maintained below about 10^{-4} mol m^{-3} (10^{-7} M), waves propagate from tip to base immediately after reactivation, but reverse after a time, which depends on the preparative technique. Using a procedure based on glycerol, tip-to-base propagation was observed for about 20 min and occasional reversal was seen during this time. Extraction and reactivation with detergent-based solutions produced waves which propagated from the tip for a period of between 2 and 4 min; no reversal was observed during this time. Electron microscopy shows that the glycerol-extracted preparations have fragments of membrane associated with the axonemal structure whereas the cells extracted with detergent have no visible membrane.

The effect of calcium ions on flagellar activity has also been investigated using ionophores, X537A and A23187, known to transport divalent ions across membranes[9]. With cells *in vivo*, the presence of either of the ionophores and 1–5 mol m^{-3} (1–5 mM) $CaCl_2$ caused the majority of organisms in a sample to propagate waves from base-to-tip—that is, in the opposite direction to that normally expected of the cells *in vivo*. When EGTA was added to the medium instead of $CaCl_2$ a few organisms propagated waves from base-to-tip but the majority maintained proximally directed waves. When ionophore, $CaCl_2$ or EGTA were added separately to a suspension of the organisms, in the concentrations used during the experiments, they caused no abnormal flagellar movements.

These results suggest strongly that calcium ions are able to control the direction of flagellar wave propagation in the preparations of *Crithidia* used. Note that the experiments described here were performed on *in vitro* systems and that the control mechanism may be different in the living cell. Nevertheless many features of the motility of the systems used are similar to those of the living organism and it is tempting to speculate that adjustment of the calcium concentration in the living cell is used to alter the direction of propagation of the flagellar waves.

The behaviour of the systems studied favours the idea that the membrane is intimately involved in the control mechanism in *Crithidia*, as suggested previously[4]. In those preparations in which the membrane is completely dissolved, namely the detergent-extracted systems, proximally directed wave propagation occurs for a few moments only after reactivation. The other systems studied are known to have residual membrane structures, although for the glycerol-extracted preparation the membrane is considerably disrupted, and show wave propagation from tip-to-base for considerable periods of time, with occasional wave reversal. A possible interpretation of these results is that in a living cell the membrane acts as a receptor for extracellular events and regulates the intra-flagellar calcium concentration, which, in turn, controls the reaction determining the direction in which waves will be propagated. An alternative explanation of the difference in behaviour between the detergent- and glycerol-extracted cells is that, in the latter case, preservation of the complete motile system is sustained for a longer period. It is well known that glycerol acts as a preservative for spermatozoa with similar axonemal structures to that of *Crithidia*, whereas detergents solubilise protein structures.

The site of calcium activity and sequestration within the flagellum is a matter for speculation at present, but a reasonable suggestion can be made with reference to the currently favoured sliding filament hypothesis[10] of flagellar activity, according to which the forces required to bend a flagellum originate in active relative sliding of the outer doublet microtubules of the axoneme. The ability to alter the direction of wave propagation in *Crithidia* implies that, for this organism at least, the relative direction of active filament sliding can be reversed. If the sliding is induced by swinging movements of linkages between the outer tubules of the flagellar axoneme it is possible that the effect of calcium is to regulate the direction in which a link will swing. If this is the case the close association of the membrane and the outer axonemal tubules would enable a stimulus originating at the membrane to be transferred quickly to the site of active force development in the organelle. It is possible that the flagellar membrane acts as the storage site for calcium ions, so that the appropriate stimulus could cause the release or sequestration of ions by the membrane, thus causing the appropriate reversal of flagellar bend movement.

Calcium has been found to have an effect on the behaviour of two other systems which carry organelles with the same 9+2 axonemal structure of the *Crithidia* flagellum. In chemically extracted *Paramecium*, ciliary reversal, which is an avoiding response *in vivo*, can be induced by raising the concentration of external calcium[2]; these observations support Naitoh's[11] view that ciliary activity in the organism is controlled by two physiological systems each of which utilises ATP. One system, activated by magnesium, is responsible for bending the cilia, whereas the other, calcium-activated, determines the direction of beating. The mechanisms of ciliary reversal could be the same as that of wave reversal in *Crithidia*, since the two activities may result from a changed sliding direction of the outer tubules. Thus, like *Paramecium*, *Crithidia* may have separate systems for propagating bends and for initiating the avoiding response, both deriving energy from ATP hydrolysis, with the former activated by magnesium and the latter by calcium.

The other system referred to is the sea urchin spermatozoa, the flagellar waveforms of which can be altered by varying the calcium concentration[12]. We have found that in addition to controlling the direction of flagellar beating, variations in the calcium concentration cause modifications in the wave shape of the flagellum of *Crithidia*. This may bear a relationship to the behaviour of sea urchin spermatozoa but we have insufficient information at present to make a detailed comparison between the two. Further experiments are in hand to quantify the effects of calcium on motility and to locate the sites of activity and sequestration of this ion within the flagellum.

We thank Roche Products Ltd, and Lilly Research Centre Ltd, for respective gifts of ionophores, X537A and A23187. This work was supported in part by a grant from the SRC. We also thank Dr M. Hughes for discussion and for assistance with the operation of the atomic absorption spectrometer.

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Received February 21; accepted March 27, 1975.

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Comparison of doubling numbers attained by cultured animal cells with life span of species

HUMAN fibroblasts in culture undergo 40–60 cell doublings (indicating numbers of cell generations) before the growth rate declines and death of cells ensues[1]. Hayflick[2] has suggested that the final doubling numbers achieved by cultured diploid cells may be related to the life span of the animal species from which the cells were derived. This suggestion is based on reported final doubling numbers for human, chicken and mouse being in the ranges 40–60, 15–35, and 14–28, whereas the maximum life span of each of these species was 110, 30 and 3.5 yr, respectively. We report here findings on final cell doubling numbers attained when cells from ten different animal species were serially subcultured. Tissues of foetal or newborn origin were used to initiate cultures from man, horse, monkey, cat and dunnart, and the cultures of wallaroo × red kangaroo hybrid, kangaroo, wallaby, rabbit and potoroo, were derived from adult tissues.

After reaching appropriate cell doubling numbers (Table 1) cell cultures derived from man, horse, wallaroo × kangaroo hybrid, kangaroo, wallaby and potoroo showed a decline in growth rate and an increase in cell granularity that could be compared with the characteristics described by Hayflick[1] as Phase III in the growth of human diploid cell strains. There was no evidence that technical procedures or infection with mycoplasmas or viruses caused this decline and final death of cultures. In the case of monkey, cat and rabbit, the cells continued to grow after the doubling numbers listed in Table 1 but counts after this time showed that there had been a shift from a diploid to a heteroploid chromosome constitution. There was no change in the growth rate or morphology of these cells at this time On last examination, the cultures from monkey, cat and rabbit had reached 100, 100 and 70 cell doublings respectively. On the other hand, the dunnart culture when last examined after 170 cell doublings had shown no change in growth rate and no change from the diploid chromosome pattern.

In our studies, the final cell doubling number for their diploid cells in the species listed cannot be related to their life spans. This applies to those which remained diploid throughout their lifetime and to those which subsequently became heteroploid. Note that these doubling numbers are based on only one culture series for each species. The final cell doubling number attained by each species may well be found to be higher when cells are cultured from a large number of individuals within one species, as experience with diploid cells cultured from a large number of human embryos has shown the final doubling number to be in the range 40–60. Adult tissues were used to initiate five of the cultures. In extensive studies on human tissues ranging in age from foetal to 90 yr, Martin *et al.*[7] showed that final doubling numbers decreased by 0.2 population doublings per year of donor life. We may therefore have obtained higher final doubling numbers for wallaroo × red kangaroo hybrid, kangaroo, wallaby, rabbit and potoroo if foetal tissue had been used, thus making the association between life

Table 1 Comparison of doubling numbers

Species	Maximum lifespan (yr)	No. of doublings attained by diploid cells	Tissue of origin of cells
Human, *Homo sapiens*	110[4]	60	Lung
Horse, *Equus caballus*	46[5]	82	Kidney
Monkey, *Macaca speciosa*	34[5]	60	Lung
Cat, *Felis cattus*	28[5]	92	Lung
Wallaroo, *Macropus robustus robustus* × Red kangaroo hybrid, *Megaleia rufa* Hybrid	19[5]	65	Skin*
Grey kangaroo, *Macropus gigantus*	16[5]	46	Skin*
Pretty-faced wallaby, *Macropus parryi*	16[5]	46	Heart*
Rabbit, *Oryctolagus cuniculus*	13[5]	70	Skin*
Potoroo, *Potorous tridactylus*	7[5]	40	Heart*
Fat-tailed dunnart, *Sminthopsis crassicaudata*	3[6]	170	Skin

* Cell lines derived from adult tissue. Other lines derived from foetal or newborn tissue. Cultures were initiated from small pieces of tissue from various sites. Growth medium was Basal Medium Eagle's (BME)[3] plus 10% foetal calf serum. Cultures from all species showed a fibroblast-like morphology, which persisted throughout the experiments, and were subcultured when confluent (within the range 4–7 d). Most cultures were divided 1:4 at subculture. Rabbit and dunnart cells grew more rapidly and could be divided 1·8 or 1:16, whereas the cultures derived from kangaroo grew more slowly and were divided 1:2. Chromosome examinations were made at regular intervals on all cell cultures to ensure that the normal diploid number of chromosomes was present.

spans of species and the final doubling numbers of their cultured cells more obscure.

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Received December 3, 1974; accepted March 27, 1975

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Heat-labile enzymes in Werner's syndrome fibroblasts

Recent reports indicate[1,2] that cultured skin fibroblasts from subjects with Werner's syndrome, a hereditary disease of premature ageing[3], have an increased proportion of heat-labile glucose-6 phosphate dehydrogenase (G-6PD) compared with normal cells undergoing ageing *in vitro*[1,2,4]. Additionally, two HL-A antigens show profoundly altered reactivities with specific antisera[2], which in total demonstrates that three diverse gene products are markedly abnormal in Werner's fibroblasts. The purpose of our study was to document further this phenomenon in Werner's cells. We now report a high heat-labile fraction of 6-phosphogluconate dehydrogenase (6-PGD), an enzyme involved in the hexose monophosphate shunt, and hypoxanthine–guanine phosphoribosyltransferase (HGPRT), an enzyme concerned with purine metabolism.

Table 1 Heat-labile fraction of enzymes in cultured fibroblasts from a subject with Werner's syndrome and controls

	% Heat-labile enzyme	
	6-Phosphogluconate dehydrogenase	Hypoxanthine–guanine phosphoribosyltransferase
Werner's syndrome		
Experiment 1	14	50
Experiment 2	24	36
Experiment 3	—	40
Controls		
Early passage	0.80±0.43 (*n* = 10, range 0–4)	7.66±1.70 (*n* = 9, range 0–14)
Late passage	4.89±1.16 (*n* = 12, range 0–9)	24.43±2.84 (*n* = 7, range 15–32)

Werner's cells were within 3–6 mean population doublings (MPD) of termination in a total replicative life span of 18 MPD; the limited number of experiments was dictated by the poor growth of these cells and finally exhaustion of their supply. Control values (mean± s.e.m.) represent experiments on strains of cultured cells from five normal donors aged 27–76 yr. Early passage cells were in the first third of their replicative life spans whereas late passage cells were within 0–9 MPD of growth termination (range of life spans 44–70 MPD). Werner's values are significantly different from late passage controls, $P < 0.01$. Controls at late passage were significantly different from early passage controls, $P < 0.01$.

Cultures were derived and propagated as described previously[2]. The assays for 6-PGD and HGPRT and the estimates of heat lability, which were accurate within 3–4%, were carried out as before[4,7]. Control strains were studied at early and late passage, the latter serving as more suitable controls for the poorly growing Werner's cells.

Table 1 indicates that normal fibroblasts accumulated an increased fraction of heat-labile 6-PGD and HGPRT during the culture life span. But Werner's cells clearly contained a higher proportion of heat-labile enzyme, even when compared to control fibroblasts at late passage. Mixing experiments with equal parts of crude extracts from Werner's and control strains gave approximately intermediate heat-labile fractions suggesting that neither proteolysis nor a deficiency of stabilising factors was responsible for the results observed in Werner's cells[1,2,4,7]. The high heat-labile fraction of HGPRT in late-passage controls considerably exceeds that of 6-PGD and previous data on G-6PD[1,2] for reasons that are uncertain but which probably relate to loss of the stabilising cofactor during the dialysis performed before HGPRT assay[7].

The data indicate that 6-PGD, an enzyme coded for by an autosomal gene and the X-linked HGPRT[7] are markedly abnormal in Werner's fibroblasts at the same time as alterations appear in G-6PD and HL-A antigens, genetic loci that are also X-linked and autosomal, respectively[6,7]. The results also show an increasingly defective fraction of 6-PGD and HGPRT in normal cells during ageing *in vitro*, as was found earlier for 6-PGD and G-6PD[1,2,4,7,8]. Indeed, similar abnormalities of the identical gene products have now been demonstrated in cultured cells derived from subjects with progeria, a related disorder of premature ageing[5-8]. The molecular nature of these defects is unclear and may even be unique in each case[7,8]. It is also not certain whether defective proteins are the consequence rather than the cause of cellular ageing. But these observations taken together with the decreased replicative potential that occurs during normal ageing[6,9,10] and the more severe growth limitation observed in Werner's and progeria fibroblasts[6,10], strengthen the view that the rate of fibroblast ageing *in vitro* is directly related to the rate of ageing *in vivo*[1,2,4-10].

This work was supported by grants from the Medical Research Council of Canada and the Canadian Diabetic Association Foundation Fund during the tenure of a scholarship from the MRC (to S.G.).

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Received January 24; accepted April 4, 1975.

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Morphine abstinence is associated with increased brain cyclic AMP

The finding that theophylline, which inhibits brain cyclic AMP phosphodiesterase[1], produces a "quasi-morphine abstinence syndrome" that is intensified by naloxone and suppressed by heroin[2] suggested that the real morphine abstinence syndrome may be associated with increased activity of a brain cyclic nucleotide. A suitable phosphodiesterase inhibitor would therefore heighten and a stimulant of phosphodiesterase would lower the intensity of real morphine abstinence signs. To test this, we administered such drugs to morphine-dependent rats shortly before withdrawal was precipitated with naloxone, so that the drugs would act only during the expression of abstinence. As these experiments indicated that the abstinence syndrome is largely associated with increased cyclic nucleotide activity, we attempted to identify the nucleotide by intracerebroventricular injection of cyclic nucleotides. These experiments indicated that cyclic AMP rather than cyclic GMP was primarily involved.

Opiate dependence was induced in male white Wistar rats (100–150 g) by a single subcutaneous injection of morphine, 150 mg kg^{-1}, in a sustained-release preparation[3]. Withdrawal was precipitated 24 h later with 0.1 or 1 mg kg^{-1} subcutaneous (s.c.) naloxone hydrochloride in 0.9% w/v saline. To modify withdrawal effects, imidazole (which stimulates phosphodiesterase[4], including that of rat brain[5]), theophylline or caffeine[1] was given orally in water 2 h before naloxone challenge; or 3-isobutyl-1-methylxanthine (IBMX), which inhibits cyclic AMP phosphodiesterase of rat brain (A. C. Roy, personal communication), guinea pig brain[6] and mouse neuroblastoma cells[7], was given subcutaneously in saline, 1 h before challenge. Alternately, cyclic AMP, cyclic GMP or the dibutyryl derivative of each, dissolved in sterile saline, was injected into the left lateral cerebral ventricle of the brain through an indwelling cannula[8], 0.5 or 1 h before challenge. After challenge with naloxone or saline, behavioural responses were recorded by observers who did not know what treatment each animal had received. Because the incidence of abstinence effects is related to the cage in which the animal is situated, rats were observed individually, either in plastic buckets, in which jumping was the predominant abstinence effect; or, for other behavioural responses, in low cages that suppressed jumping. Jumps were counted, and other withdrawal signs were recorded as present or absent. The significance of differences between jumping scores, and between total scores for the abstinence syndrome as a whole, were tested by the Mann–Whitney *U* test[9], adapted for the computer[10]. For quantal counts, Fisher's Exact Probability Test[9] was used.

As jumping was a characteristic and readily quantifiable sign of opiate abstinence, we tested how far drugs that modify brain phosphodiesterase activity affected the jumping of dependent rats when abstinence was precipitated with the higher dose of naloxone (1.0 mg kg^{-1}). Table 1 shows that much jumping occurred in rats that had received sustained-release morphine, followed by a phosphodiesterase inhibitor and naloxone. When either morphine or phosphodiesterase inhibitor was omitted from the treatment schedule, jumping was markedly reduced. Imidazole, in dependent rats, significantly decreased jumping and overcame the ability of theophylline to increase jumping.

These results suggested that this expression of abstinence was associated with an increase in brain cyclic nucleotide. To identify the nucleotide, we administered cyclic AMP, cyclic GMP or one of their dibutyryl derivatives into the left lateral cerebral ventricle of morphine-dependent rats and observed jumping after precipitating abstinence with naloxone (1.0 mg kg^{-1}). Intracerebroventricular injection of cyclic AMP and, to a lesser extent, dibutyryl cyclic AMP significantly increased jumping (Table 1). Cyclic GMP did not do so, although dibutyryl cyclic GMP increased jumping to an extent that approached statistical significance ($P = 0.052$). Dibutyryl cyclic GMP, however, significantly decreased two abstinence signs, teeth chattering ($P < 0.05$) and chewing ($P < 0.01$). That these were the two signs that IBMX also significantly lessened at 5–7 min after challenge (see below) suggested that

Table 1 Effects of cyclic nucleotides or of drugs inhibiting or stimulating phosphodiesterase on naloxone-precipitated jumping in morphine-dependent rats

Treatment				Jumping responses		
0 h	22 h	23 h	No. of rats	Rats jumping (%)	Mean no. of jumps	Median no. of jumps
Vehicle	Th, 100	—	10	10	0.4	0
Mo, 150	Vehicle	—	39	87	7.4	4.0
Mo, 150	Th, 100	—	38	100*‖	60.5	49.0‡‖
Mo, 150	Vehicle	—	11	82	4.9	4.0
Mo, 150	Th, 25	—	10	100	17.7	14.5*
Mo, 150	Th, 50	—	11	100	36.6	33.0†
Mo, 150	Vehicle	—	10	80	3.5	2.0
Mo, 150	Cf, 25	—	10	100	10.1	3.5
Mo, 150	Cf, 50	—	11	100	10.4	12.0†
Mo, 150	Cf, 100	—	10	100	22.6	12.0‡
Vehicle	—	IBMX, 10	10	30	2.3	0
Mo, 150	—	Vehicle	8	63	3.6	2.0
Mo, 150	—	IBMX, 2.5	8	88	11.6	5.5
Mo, 150	—	IBMX, 5.0	8	88	15.4	8.5
Mo, 150	—	IBMX, 10.0	8	100¶	44.0	27.5‡‖
Mo, 150	Vehicle	—	10	100	8.8	6.5
Mo, 150	Im, 250	—	10	60	3.3	1.5*
Mo, 150	Th, 100	—	10	100	63.0	46.5
Mo, 150	Th, 100+Im, 250	—	11	100	19.9	11.0†
Mo, 150	—	Vehicle	16	50	1.75	0.5
Mo, 150	—	cAMP, 50	18	83*	8.17	4.0†
Mo, 150	—	Vehicle	11	87	3.5	1.0
Mo, 150	—	dbcAMP, 25	13	92	8.5	8.0*
Mo, 150	—	Vehicle	36	58	2.9	1.0
Mo, 150	—	dbcAMP, 50	34	61	5.6	1.0
Mo, 150	—	Vehicle	25	52	4.1	1.0
Mo, 150	—	cGMP, 50	26	69	4.1	3.0
Mo, 150	—	Vehicle	34	50	3.6	0.5
Mo, 150	—	dbcGMP, 50	36	67	5.0	2.0

At 24 h every rat received naloxone (1.0 mg kg^{-1} s.c.) in saline. Values for jumping responses are based on the count for 15 min immediately after naloxone challenge.

Mo, sustained-release subcutaneous morphine; Th, theophylline given orally in water; Cf, caffeine given orally in water; IBMX, 3-isobutyl-1-methylxanthine subcutaneously in saline; Im, imidazole given orally in water; —, no treatment. Oral or subcutaneous drug doses in mg per kg of base. cAMP, cyclic AMP; dbcAMP, dibutyryl cyclic AMP; cGMP, cyclic GMP; dbcGMP, dibutyryl cyclic GMP; given through an indwelling polyethylene cannula into the left lateral cerebral ventricle. Intracerebroventricular drug doses in μg per rat.

P values for the jumping scores were calculated by the Mann-Whitney *U* Test. For comparisons of test drug with vehicle in morphine-dependent rats: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. For comparisons of test drug in rats treated or not treated with morphine, §$P < 0.05$, ¶$P < 0.01$, ‖$P < 0.001$.

Table 2 Intensification by IBMX or cyclic AMP of the abstinence syndrome in morphine-dependent rats

Response	% Incidence of responses after treatment schedule				
		Experiment with IBMX		Experiments with cyclic AMP	
	(a) V+I+N	(b) M+V+N	(c) M+I+N	(d) M+V+N	(e) M+A+N
Jumping	NT	NT	NT	NT	NT
Teeth chattering	0	0	6.7	100	87
Irritable to touch	60	67	100^{ab}	43	74^{d}
Irritable to handling	33	47	100^{ab}	67	78
Diarrhoea	0	40^{a}	53^{a}	52	65
Chewing	33	33	33	90	87
Ptosis	53	53	73	24	52
Body shakes	0	0	6.7	38	61
Head shakes	0	0	0	10	48^{d}
Paw tremor	0	0	27^{ab}	10	22
Rearing	0	13	60^{ab}	100	100
Restlessness	6.7	0	40^{ab}	62	78
Salivation	0	0	27^{ab}	0	0
Ejaculation	NT	NT	NT	43	78^{d}
Median total score*	2	3	5^{ab}	6	8^{d}

All rats were treated subcutaneously with 150 mg kg^{-1} morphine (M) in a sustained-release preparation, or with vehicle (V) and, 24 h later, all were challenged with naloxone (N; 0.1 mg kg^{-1} subcutaneously). In the experiment with IBMX, three groups, each of 15 rats, received 1 h before challenge, either IBMX (I; 10 mg kg^{-1} s.c.) or vehicle, and responses were recorded during 15–17 min after challenge. In the experiments with cyclic nucleotides, groups totalling 21 and 23 rats received respectively into the left lateral cerebral ventricle, 0.5 h before challenge, either saline (V) or 50 µg cyclic AMP (A) and responses were recorded during 15 min after challenge.

Letters a–e in the body of the table indicate comparisons between columns where a significantly greater incidence was found; where the letter is italic, $P < 0.01$; where it is not, $P < 0.05$.

*The significance of the difference between intensities of the abstinence syndrome as a whole was based on the median total scores.

NT, Not tested or not recorded.

some increase in cyclic GMP, induced by inhibition of phosphodiesterase, may be part of the pattern of the quasi-abstinence syndrome, but that cyclic GMP contributes little to the real abstinence syndrome, in which both of these signs are prominent (Table 2, column d).

Certain drugs, such as atropine, which interfere with neurotransmitters inhibit some abstinence signs in the rat, but intensify others, whereas heroin lessens the incidence of all signs[3]. To determine how closely the substances under investigation were related to the generation of the abstinence syndrome as a whole, we studied the extent these substances affected other abstinence signs, as well as jumping. Three groups, each of 15 rats, received either sustained-release morphine or vehicle; 23 h later each group received IBMX or vehicle; and at 24 h, all were challenged with the lower dose of naloxone (0.1 mg kg^{-1}), and the presence of abstinence signs was recorded during two 2 min observation periods. When responses were recorded 5–7 min after challenge, irritability to touch ($P < 0.05$) and handling ($P < 0.001$), rearing ($P < 0.001$) and restlessness ($P < 0.001$) were significantly increased by IBMX; but teeth chattering ($P < 0.05$) and chewing ($P < 0.025$) were significantly reduced. At 15–17 min after challenge, however, six of the twelve individual abstinence signs were significantly increased by IBMX (Table 2, columns b and c). IBMX also increased the intensity of the abstinence syndrome as a whole ($P < 0.002$).

In two experiments, to test whether cyclic AMP increased other abstinence signs, as well as jumping, rats received sustained-release morphine and 23.5 h later, an intracerebroventricular injection of either 50 µg cyclic AMP or saline. Half an hour later, all rats were challenged with 0.1 mg kg^{-1} naloxone and the occurrence of behavioural responses, other than jumping, was recorded. The incidence of body shakes ($P < 0.05$) and head shakes ($P < 0.025$) was significantly greater after cyclic AMP (ten rats) than after saline (nine rats). In a second experiment, the incidence of ptosis ($P < 0.025$) and of ejaculation ($P < 0.05$) was significantly greater in the cyclic AMP group (13) than in the saline group (12). None of the responses was significantly greater in the saline controls in either experiment. Table 2 (columns d and e) gives the pooled results of these two experiments, in which irritability to touch was also significantly greater in the rats receiving cyclic AMP. In these rats, also, the intensity of the abstinence syndrome as a whole was significantly greater ($P < 0.001$). In the first of these two experiments, a third group (nine rats) showed that intracerebroventricular cyclic GMP was no more effective than saline. After cyclic AMP, the abstinence syndrome as a whole was more intense ($P < 0.05$), and the incidence of body shakes ($P < 0.005$) and of ejaculation ($P < 0.05$) was greater, than after cyclic GMP.

As inhibitors of phosphodiesterase intensified abstinence effects, we tested whether naloxone inhibited this enzyme. At concentrations up to mmol l^{-1}, however, naloxone did not inhibit cyclic AMP phosphodiesterase in rat brain homogenate (A. C. Roy, personal communication).

Our experiments show that drugs modifying the activity of brain cyclic AMP phosphodiesterase largely modify inversely the intensity of morphine abstinence effects. Thus the intensity of such effects can probably be associated with the level of cyclic nucleotide in brain cells. Injection of cyclic AMP intracerebroventricularly intensified several abstinence signs, without significantly diminishing any, whereas cyclic GMP reduced some of these signs, without significantly increasing any. Moreover, cyclic AMP intensified the abstinence syndrome as a whole, but cyclic GMP did not. These results argue that increase in cyclic AMP in appropriate brain neurones is associated with the generation of the morphine abstinence syndrome.

Reports on the relationship of abstinence effects to cyclic AMP in precipitated opiate withdrawal are consistent with our findings. Thus, a direct association was observed between whole brain cyclic AMP content and the intensity of the precipitated abstinence syndrome in morphine-dependent rats[11]. Also, cyclic AMP, administered to dependent mice, intensified the weight loss, although not the jumping, of precipitated abstinence[12]. These observations, coupled with our experimental findings suggest that opiate dependence is a state of potential increase in activity of a neuronal cyclic AMP mechanism, held in check by the presence of opiate, which inhibits an adenyl cyclase of morphine-sensitive neurones[13-15].

We thank Dr W. A. Klee, Mr A. C. Roy, Dr S. A. Saeed and Mr C. Schneider for helpful discussions; Mr M. A. Collins, Mr N. J. Cuthbert, Miss J. F. de C. Sutherland, Miss L. A. Taylor and Mr C. M. Tobin for observing animals, Mr L. C. Dinneen for statistical advice and Sankyo Limited for naloxone.

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Received December 16, 1974; accepted April 4, 1975.

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Impulse-flow regulation of high affinity choline uptake in brain cholinergic nerve terminals

ACETYLCHOLINE (ACh) synthesis is coupled, in some unknown manner, to ACh release caused by neuronal impulse flow[1–4]. Accordingly, several models have been proposed regarding the regulation of ACh synthesis. These include feedback competitive inhibition of choline acetyltransferase by ACh[5,6], and mass action regulation of choline acetyltransferase[7]. More recently, high affinity choline uptake, which is very localised to cholinergic nerve terminals[8], has been proposed as a regulatory step in ACh synthesis[9–12]. If high affinity choline uptake were coupled to ACh synthesis and release, then one would expect changes in the high affinity choline uptake system after alterations in synthesis and release. We have found changes in the high affinity choline uptake system in hippocampal synaptosomes after treatments which alter neuronal impulse flow to cholinergic nerve terminals.

The rat hippocampus was used because of its known cholinergic innervation from the septum[8], and because of the possibility of altering neuronal impulse flow in this tract[4,13]. We used two means of slowing and/or stopping impulse flow. These were intraperitoneal injection of pentobarbital, and placement of acute radio frequency lesions in the septum under ether anaesthesia[13,14]. The evidence that pentobarbital stops or slows impulse flow has been summarised and supported recently[13]. Placement of acute lesions would stop impulse flow by interrupting the axons. In pentobarbital-treated animals we have reversed the cessation of impulse flow by electrical stimulation of the medial septal area[4].

A kinetic analysis of the high affinity choline uptake into hippocampal synaptosomes from untreated control animals revealed K_m and V_{max} values of about 0.8 μM and 60 pmol per 4 min per mg protein respectively (Fig. 1, Table 1). These values are similar to those obtained by other workers[9–11,15]. A similar analysis using synaptosomes from animals pretreated with pentobarbital (30 min) or septal lesions (1 h) revealed a decreased V_{max} (40%) with no change in the apparent K_m. When impulse flow to the hippocampus was restored in pentobarbital-treated animals by stimulating the septum for 10 min, the pentobarbital-induced reduction in V_{max} was reversed and the kinetic parameters returned to untreated control values (Fig 1, Table 1). These kinetic changes were observed in every experiment (range of reduction in V_{max} in individual experiments was 30–65%). Complementary to the above changes, we have found that administration of scopolamine, an anticholinergic drug which would increase ACh turnover, and pentylenetetrazol, a central nervous system stimulant, results in a 25–50% increase in choline uptake.

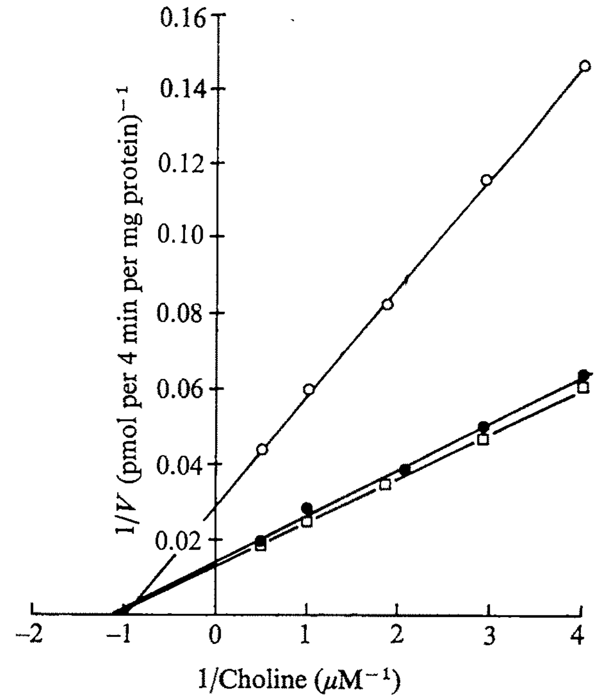

Fig. 1 Lineweaver–Burke analysis of high affinity choline uptake into hippocampal synaptosomes from untreated control rats, pentobarbital (65 mg kg^{-1}) treated rats, and electrically stimulated pentobarbital-treated rats. Male Sprague-Dawley rats (175–225 g) were administered pentobarbital intraperitoneally and decapitated 30 min later. Electrical stimulation[4] (40 Hz, 200 μA) was begun 20 min after injection, and continued for 10 min. Animals were decapitated 7–10 s later. In all experiments, the hippocampus (whole, bilateral hippocampal formations) was dissected rapidly and homogenised in 20 volumes of 0.32 M sucrose. Crude mitochondrial fractions were isolated by differential centrifugation and resuspended in the original volume of 0.32 M sucrose. Samples (100 μl) of this suspension were added to 0.9 ml of Krebs–Ringer solution, and incubated at 30° C for 4 min for uptake measurements[12,14]. Blank values were obtained by maintaining parallel samples at 0–2 °C. Choline concentrations added to the incubation media varied from 0.2 to 2 μM. Higher concentrations were not used because uptake by the low affinity component would tend to predominate[15]. Lines were fitted to the data points by least squares analysis. ○, Pentobarbital; ●, control; □, pentobarbital + stimulation.

No inhibition of uptake into synaptosomes from untreated animals was observed when 10^{-3} M pentobarbital was added directly to the incubation medium during the uptake process. Administration of pentobarbital to rats had no effect on the uptake of low concentrations of ^{3}H-glutamate or ^{3}H-tyrosine by hippocampal synaptosomes, indicating that the reduction in choline uptake was not a nonspecific effect on synaptosomal uptake in general. Since there are large post-mortem increases in choline levels[16], it seemed possible that the changes in V_{max} observed here could have been caused by isotopic dilution of labelled choline by endogenous choline, which may be produced to a greater extent in pentobarbital-treated and lesioned animals than in controls. When endogenous choline levels in the media were measured after incubation, however, they were extremely low and no difference was found between synaptosomal fractions from pentobarbital-treated animals and those from control animals. It also seemed possible that our results could have been caused by an increased efflux of ^{3}H-choline or ^{3}H-ACh from synaptosomes from treated animals, rather than by a decreased uptake. But, the efflux of radioactivity from resuspended, prelabelled synaptosomes (prepared for routine uptake) from treated or control animals showed no differences. The reversal of the pentobarbital effect on V_{max} by stimulation was restricted to the hippocampus, and occured in no other brain regions. This indicates that the reversal was dependent on anatomical connections for carrying the impulses and was not caused by nonspecific effects of surgery.

The effect of pentobarbital administration on the low

Table 1 Alterations in high affinity choline uptake into rat hippocampal synaptosomes

Treatment	K_m(μM)	V_{max} (pmols per 4 min per mg protein)
None	0.76 ± 0.08 (5)	59.8 ± 4.0 (5)
Acute septal lesion	0.84 ± p.04 (4)‡	35.8 ± 24 (4)*
Pentobarbital	0.95 ± 0.08 (5)‡	38.4 ± 2.9 (5)†
Pentobarbital + septal stimulation	0.75 ± 0.03 (5)‡	59.6 ± 3.6 (5)‡

See legend to Fig. 1 for experimental details. Values are mean ± s.e.m. (*n* = number of experiments.)

* 40% reduction compared with untreated controls, $P < 0.05$.

† 36% reduction compared with untreated controls, $P < 0.025$.

‡ No significant difference compared with untreated controls.

Acute septal lesions were placed under ether anaesthesia so that anaesthetic was not present at time of death. Sham lesioned animals did not have a reduced synaptosomal uptake of choline.

affinity uptake[15] of choline was also examined. With high concentrations of ^{3}H-choline in the medium (20–150 μM), when most uptake would be by the low affinity system[15], only a slight decrease (5–10%) in uptake velocity was observed in hippocampal synaptosomes derived from pentobarbital-treated animals. This change, however, could be explained on the basis of the presence of the high affinity uptake component, which would still account for a significant fraction of the choline accumulated, even at these higher concentrations[15]. Thus, these impulse flow-dependent changes in choline uptake are probably restricted to the high affinity system which is highly localised to cholinergic nerve terminals[8].

While these data demonstrate an impulse-flow regulation of the accumulation of choline by cholinergic terminals, it is difficult to distinguish if the impulse-flow regulation acts primarily on the uptake mechanism or secondarily after some other regulatory change. This is because one cannot clearly separate choline uptake from subsequent acetylation of choline, that is, there is no inhibitory drug or simple treatment to block acetylation without also inhibiting uptake On the other hand, uptake seems to be primarily affected. We have examined choline acetyltransferase in the absence of uptake, that is, in its soluble state, and have found no change in its kinetic properties with regard to substrate or cofactor This situation is in contrast to that with the dopamine-containing neurones where kinetic changes are found in soluble tyrosine hydroxylase after changes in impulse flow[17]. Also, when we examined the synaptosomal content of ^{3}H-choline and synthesised ^{3}H-ACh from control and treated animals, we found a similar ratio of these componds in both cases. Thus the reduction in uptake after treatment could not be explained solely by reduced conversion of choline to ACh. Also, the reduction in uptake after treatment is only observed when synaptosomes are incubated in the presence of sodium ion; this reflects the involvement of the high affinity uptake system which is highly sodium dependent[9,15].

Since the molecular mechanisms underlying choline uptake are unknown, we do not know how the observed changes in V_{max} occur. Because the change is in uptake velocity rather than affinity, it may be that the net result of reducing impulse flow is to either partially immobilise or slow the presumed choline carrier. Since choline uptake is dependent on ions, most notably sodium[9,15], changes in ionic fluxes across the membrane during impulse flow may in some way be responsible for the regulatory influence.

The results presented here show that changes occur in the high affinity choline uptake system after treatments which alter the activity of cholingeric neurones. The reduction in V_{max} in conditions where impulse flow is presumably stopped or slowed, and its increase after stimulation, would have functional significance in that the high affinity choline uptake system would respond to the need of the neurone for formation of releasible ACh. This possibility receives additional support from experiments with perfused ganglia where nerve stimulation accelerates choline uptake by cholinergic terminals[18,19]. These results provide strong support for the view that choline uptake is rate-limiting[9–12] and regulatory in the synthesis of ACh.

This research was supported by grants from the US Public Health Service and the John A. Hartford Foundation.

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Dopamine-like effects of cholera toxin in the central nervous system

IT is now well established that cyclic AMP acts as an intracellular 'second messenger' in mediating the effects of several hormones on their target cells[1]. The hypothesis that cyclic AMP serves a similar function in mediating the effects of certain neurotransmitters in the central nervous system (CNS) has received some support[2,3]. For example, the neurotransmitters noradrenaline and dopamine stimulate the synthesis of cyclic AMP in tissue slices or homogenates of brain regions innervated by neurones which use these transmitters[4–6]. Further, the actions of these transmitters on neural firing when applied by iontophoresis can be mimicked by cyclic AMP[2]. Bilateral injections of small amounts of dopamine into the nucleus accumbens of rats stimulates locomotor activity[8]. In the present experiments small amounts of cholera toxin were injected bilaterally into the nucleus accumbens of rats and the time course of its effects on adenylate cyclase and locomotor activity were determined. The only known mechanism of action of cholera toxin (choleragen) is activation of adenyl cyclase[7] in intact cells subsequent to its binding to a cell-surface receptor, the ganglioside GM$_1$ (refs 9–11). The results support the hypothesis that dopaminergic transmission in the CNS is mediated by cyclic AMP.

Cannulae were implanted bilaterally into the nucleus accumbens septi (NAS) of adult male Sprague–Dawley rats and choleragen (1 μg in 1μl) or 1 μl of vehicle was injected. To distinguish between possible pre- or postsynaptic effects of the toxin on dopaminergic mechanisms, a further group of animals was prepared which had selective bilateral lesions of the dopamine terminals in the nucleus accumbens induced

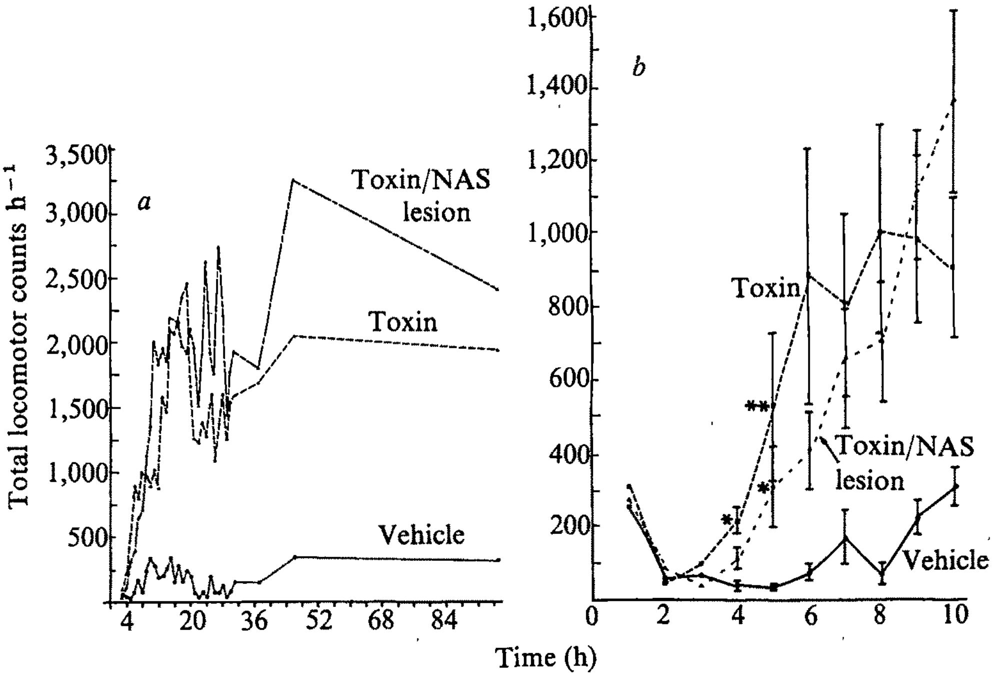


Fig. 1 *a*, Effect of choleragen on locomotor activity. Three groups of six adult male Sprague–Dawley albino rats were used. One group (Toxin) received bilateral injections of choleragen (1 μg in 1 μl) into the nucleus accumbens. Another group (Vehicle) was similarly treated with injections of vehicle solution (0.125 M Tris-HCl, *p*H 7.4, containing 0.05 M NaCl and 0.00025 M EDTA). A third group (Toxin/NAS lesion) had been previously treated with 6-hydroxydopamine (8 μg in 2 μl) injected one month previously bilaterally into the nucleus accumbens to destroy the dopaminergic innervation before toxin treatment. Toxin was prepared according to the method of Finkelstein and Lo Spalluto[15]. Injections of choleragen or vehicle were made at a rate of 1 μl min^{-1} through 30 gauge stainless steel tubes inserted into chronically implanted guide cannulae (23 gauge stainless steel) which had previously been stereotaxically implanted in the NAS. Locomotor activity was measured in a bank of wire cages each with two horizontal photocell beams along the long axis. One animal was placed in each cage. The number of photocell beam interruptions per hour was recorded at hourly intervals for 30 h. Thereafter the animals were replaced in their home cages. Locomotor activity was recorded intermittently for a further period of 14 days. *b*, Shows an expansion of the first 10 h of (*a*). Locomotor activity in the toxin-treated groups becomes significantly activated above control activity at the fourth and fifth hours after choleragen injection. $^{*}P<0.05$, $^{**}P<0.01$.

by 6-hydroxydopamine injections (8 μg in 2 μl) one month before choleragen administration. This procedure reduces nucleus accumbens dopamine concentration to approximately 20% of control, whereas striatal dopamine is not affected (P. H. K., P. Seviour, and S. D. Iversen, in the press). These animals also had cannulae bilaterally implanted and received choleragen (1 μg in 1 μl) bilaterally After injection rats were placed in individual photocell cages and locomotor activity recorded every hour for the first 36 h and then at periodic intervals. No effect was seen for the first 3 h, after which time locomotor activity in the lesioned and non-lesioned groups of animals receiving choleragen rapidly increased to levels approximately ten times greater than those observed in control animals receiving vehicle injections (Fig. 1). Locomotor stimulation was still present 4 d after the injection. In fact some animals still showed significantly elevated locomotor activity 2 weeks after choleragen injection. As the locomotor activity seen in lesioned animals is not significantly different from normal animals receiving choleragen it is probable that the observed effects are postsynaptic.

It is possible to see activation of adenyl cyclase in disrupted membranes from cells previously treated with choleragen[12]. Adenyl cyclase activity was therefore measured in homogenates of NAS of rats previously treated with choleragen (Fig. 2). After 2 h the activity of adenyl cyclase in accumbens homogenates was not significantly different from that in vehicle-treated animals. At 5 h, however, when stimulation of locomotor activity was apparent, adenyl cyclase activity was also elevated. Moreover, at 24, 48 and 96 h after choleragen, when locomotor activity was further stimulated, adenyl cyclase activity was also further increased. Thus the effects of choleragen on locomotor activity and adenyl cyclase activity exhibited similar time courses, showing in each case a characteristic lag period, similar to that observed in other cells after exposure to toxin[12]—for example, stimulation of lipolysis in isolated adipocytes by choleragen shows a lag of approximately 90 min[12]. The locomotor effects after choleragen contrast with those observed after dopamine injection into the nucleus accumbens, when locomotor stimulation is immediately apparent and persists for only about 5 h[8]. As mentioned

Table 1 Adenyl cyclase activity (pmol per mg protein in 2.5 min) in different brain areas of control and toxin-treated rats at different times

	Control			Toxin		
Area	5 h	48 h	96 h	5 h	48 h	96 h
Striatum	206,178	—	106,188	108,172	—	388,564
Olfactory tubercle	—	210,168	—	—	396,466	—
Hypothalamus	112,138	—	116,152	128,158	—	88,138
Cortex	128,146	—	162,156	122,162	—	118,202

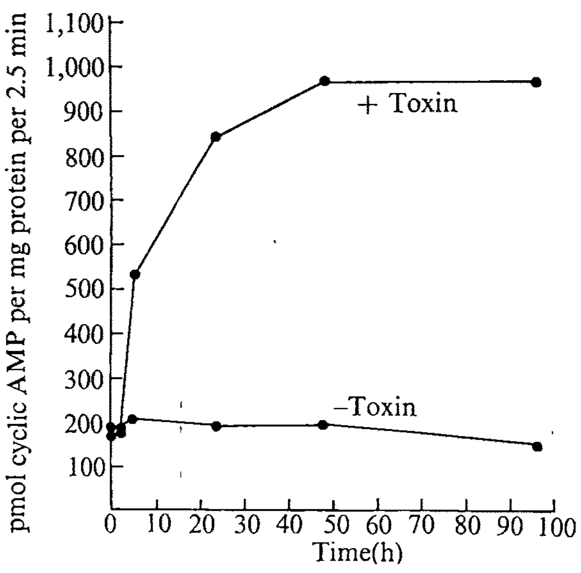
Toxin (1 μg μl^{-1}) or vehicle was injected bilaterally into the nucleus accumbens. Each figure represents one animal (duplicate determinations). Striata, olfactory tubercle and cortex were pooled in each animal. Dissections were carried out according to the methods of Glowinski and Iversen[18] and Horn *et al.*[13]. Adenyl cyclase was assayed as described previously[13].

above, the effects of choleragen in some animals lasted for up to 2 weeks. The duration of the effect may be related to the turnover time of adenyl cyclase molecules in the neuronal membrane Other effects, such as an induction of phosphodiesterase postsynaptically, cannot be ruled out.

We also investigated the effects of added dopamine on adenyl cyclase in nucleus accumbens homogenates from toxin-treated animals (Fig. 3). As previously reported[13], dopamine (10^{-4} M) approximately doubled adenyl cyclase activity when added to nucleus accumbens homogenates from vehicle-treated rats. Two hours after toxin treatment the effect of dopamine did not differ from that in control animals. After 5 h, however, when both locomotor activity and adenyl cyclase activity were stimulated, dopamine was able to increase the adenyl cyclase activity still further. The absolute increase in adenyl cyclase activity produced by 10^{-4} M dopamine 5 h after toxin was significantly greater than the increase produced by 10^{-4} M dopamine after 2 h or in control animals. A similar phenomenon has been reported by Field[14] who found that the addition of noradrenaline stimulated adenyl cyclase both in normal erythrocytes and in cells when adenyl cyclase activity had been increased by previous exposure to choleragen The absolute increase in enzyme activity found with noradrenaline after choleragen treatment was greater than that found in normal cells After 24 h, however, addition of dopamine failed to stimulate adenyl cyclase in nucleus accumbens homogenates above toxin-stimulated levels. Various explanations are possible for this phenomenon. By this time, for example, a maximal stimulation of adenyl cyclase activity in the heterogeneous cell population may mask the increase caused by dopamine. Alternatively dopamine may not further stimulate adenyl cyclase if this enzyme is already maximally activated It is also possible that long term stimulation of postsynaptic dopamine receptive neurones by choleragen leads to a reciprocal desentisation of the postsynaptic dopamine receptors.

To determine how closely the activation of adenyl cyclase in the nucleus accumbens correlated with locomotor stimulation, adenyl cyclase was assayed in other brain regions at various times after choleragen injections (Table 1). Adenyl cyclase activity in homogenates of hypothalamus and cerebral cortex was unchanged at 5 and 96 h; 48 h after injection, however, the olfactory tubercle showed increased activity and at 96 h the striatum did as well. Nevertheless, since locomotor stimulation was seen as early as 4 h after toxin injection it seems likely that activation of adenyl cyclase in the nucleus accumbens correlates best with the behavioural response. Rectal temperature was not increased in toxin-treated rats up to 96 h.

Fig. 2 Effect of choleragen (1 μg in 1 μl) injected bilaterally into the nucleus accumbens on adenyl cyclase activity in homogenates of this brain region at various times after the injection. The nucleus accumbens was dissected out according to the method of Horn *et al.*[13] and adenyl cyclase activity measured as described previously[13]. Points are means of at least three separate incubations of at least four separate nuclei accumbens.



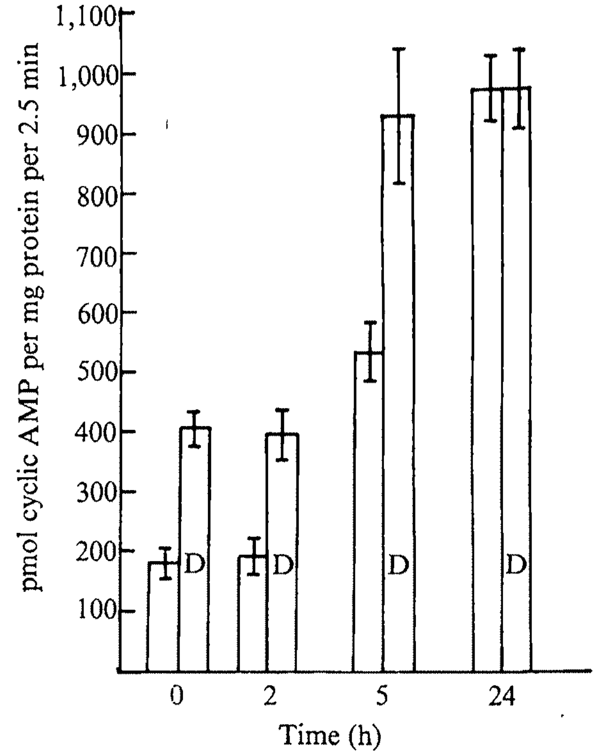


Fig. 3 Effect of dopamine (10^{-4} M) on adenyl cyclase in homogenates of the nucleus accumbens of rats pretreated with bilateral injections of choleragen (1 μg in 1 μl) at various times after the injection Adenyl cyclase assays were carried out as in Fig. 2, except that some assay tubes contained 10^{-4} M dopamine (D). Values represent means ±s.e.m. of at least duplicate incubations of tissue from four or more animals. The increase in adenyl cyclase observed at 5 h with dopamine is significantly greater than that observed at 0 or 2 h. ($P<0.01$.)

It has been postulated that dopaminergic transmission is mediated by cyclic AMP. The present studies demonstrate that a dopamine-mediated behavioural syndrome in rats may be mimicked by activating adenylate cyclase at the same locus, thus bypassing the dopamine receptors. The behavioural effects and the adenyl cyclase activation have the same temporal characteristics. It also seems possible that cholera toxin will be a useful tool for studying other cyclic AMP-mediated phenomena in the CNS.

We thank Drs L. L. and S. D. Iversen and Mrs J. Reed for assistant. Coleragen was given by Prof. R. Finkelstein and Dr C Miller R. J. M. is an MRC Scholar. P. H. K. acknowledges his ICI Postdoctoral Fellowship

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Domoic and quisqualic acids as potent amino acid excitants of frog and rat spinal neurones

THE finding that kainic acid (Fig. 1*a*) is a potent glutamate-like excitant of rat cerebral[1] and cat spinal[2] neurones has led us to test domoic acid (Fig. 1*b*), which is structurally related to kainic acid and which also occurs in certain marine algae and has similar anthelmintic properties[3,4]. We have also compared the actions of these two compounds with those of α-allo-kainic acid (Fig. 1*c*), a stereo-isomer of kainic acid and a weaker excitant of rat cerebral neurones[1], and with quisqualic acid (Fig. 1*d*), an ascaricidal compound isolated from seeds of the plant genus *Quisqualis*[5]. Quisqualic acid is highly potent as a glutamate agonist at the crayfish neuromuscular junction[6]. Compounds were tested on rat spinal interneurones by microelectrophoresis[7] and on frog spinal motoneurones by superfusion of the procaine-blocked[8] hemisected spinal cord *in vitro*[9]. On both these preparations (see Table 1), the anions of domoic and quisqualic acids were found to be at least two orders of magnitude more potent than L-glutamate, and equal to or stronger than kainate, whereas α-allo-kainate was a considerably weaker excitant.

The high activity of quisqualate extends the range of structures which can replace the terminal carboxymethyl group of L-glutamate with enhancement of activity. These alternative terminal groups include those given in Table 2. All of these acidic terminals contain additional electronegative atoms or groups which may promote an increased interaction of the terminal group as a whole with a corresponding electropositive region of the receptor. The high potency of quisqualic acid (one to two orders higher than that of any of the other compounds of Table 2) indicates an exceptionally effective interaction of the isoxazolidinedione moiety with this electropositive receptor site. The equally potent actions of domoate and kainate may have a similar explanation. Although these two substances have the same carboxymethyl terminal as glutamate, the considerably weaker action of α-allo-kainate, and also of dihydrokainate[2], indicates that the high activity of kainate and domoate is associated with unsaturation in the side chain coupled with a *cis* relationship of the C_3 and C_4 substituents. This suggests the possibility of synergistic action of the side chain unsaturation and the carboxymethyl terminal in the interaction of these parts of the molecule with the electropositive region of the receptor.

When allowance is made for the different experimental conditions used, the high potency of domoate, quisqualate and kainate in depolarising spinal neurones seems to compare with that of certain peptides related to substance P (refs 16 and 17). It is not known, however, whether the amino acids and these peptides act at the same sites. The amino acids are all closely related structurally to L-glutamic acid, and it seems likely that they act on glutamate receptor sites. Whether these are identical with the receptor sites for synaptically-released

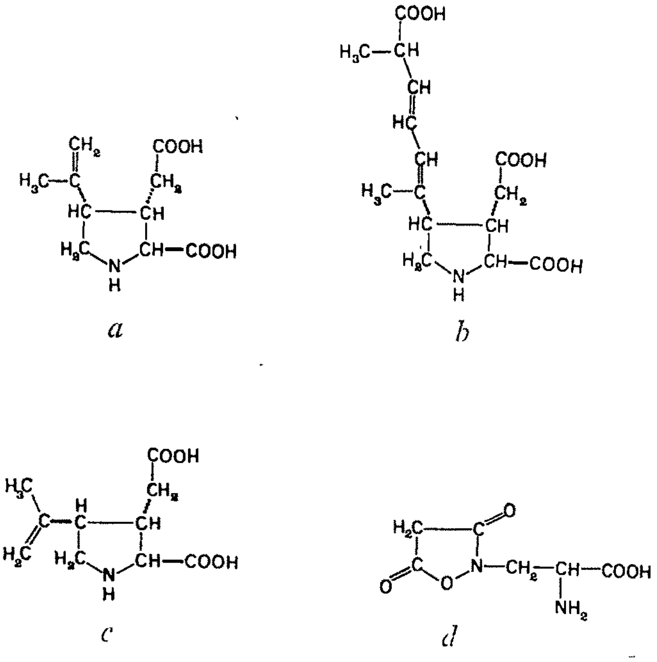


Fig. 1 Structures of kainic acid (*a*), domoic acid (*b*), α-allo-kainic acid (*c*) and quisqualic acid (*d*).

Table 1 Relative potency of amino acid excitants

Compound	Potency relative to L-glutamate = 1.0	
	Rat spinal interneurones*	Frog spinal motoneurones‡
α-allo-Kainate	1–3	0.4 (0.3–0.5; 5)
DL-Homocysteate	3–5	11 (6–14; 10)§
Kainate	40–>200†	127 (85–185; 19)
Quisqualate	25–>200†	389 (151–614; 10)
Domoate	50–>200†	293 (247–373: 10)

For microelectrophoretic administration, domoic, quisqualic and kainic acids were used as the monosodium salts (20 mM) dissolved in 150 mM NaCl, and α-allo-kainic acid as the monosodium salt (200 mM) in water. Monosodium L-glutamate (200 mM in water) and monosodium DL-homocysteate (20 mM in 150 mM NaCl or 200 mM in water) were used as standard excitants. Extracellular recordings of the discharge of rat lumbar spinal interneurones were made using the 4 M NaCl-filled centre barrel of seven-barrel micropipettes. Full details of the preparation have been described[7]. Comparisons of drug potency were made by increasing the current required in each barrel to produce equal frequencies of spike discharge. The superfusion solution used for the frog spinal cord experiments had the following composition: NaCl, 111 mM; KCl, 2 mM; NaH_2PO_4, 1mM; $CaCl_2$, 2 mM; Tris base, 10 mM; $NaHCO_3$, 10 mM; glucose, 12 mM; procaine hydrochloride, 1 mM; and 11.3 M HCl was used to adjust the solution to *p*H 7.9. The temperature was maintained at 13 °C. The effect of a compound added to the solution was measured from the magnitude of the potential recorded between the distal end of ventral roots 8 or 9 and the bathing solution. This potential was generated by changes in motoneurone membrane potential, electrotonically propagated along the ventral root[10]. Procaine was included in the perfusion medium to block conduction in the preparation, thereby preventing the indirect effects of the test substances on motoneurones which could otherwise arise through actions on interneurones. Tetrodotoxin (10^{-7} M) was also used for this purpose in some experiments, and gave identical results. The test substances all caused motoneuronal depolarisation. Dose-response curves were obtained by the addition of the substances to the medium in various concentrations.

*Microelectrophoretic application. Inverse ratios of ejection currents required to achieve equal firing frequencies. Multiple comparisons on eight interneurones in two animals.

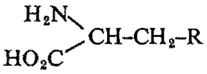
†In some instances, merely reducing the retaining currents for the three strongest excitants was sufficient to achieve a firing rate equal to that obtained by 20–60 nA L-glutamate (200 mM) or of DL-homocysteate (20 mM in 150 mM saline). Only minimum potency ratios could be calculated in these cases.

‡Calculated by comparison of ventral root depolarisation caused by various concentrations of excitants with the log dose–response curve for L-glutamate. Comparisons made on three frog cords; range and number of observations in parentheses.

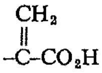
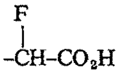
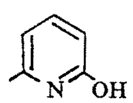
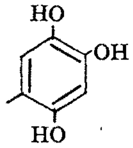
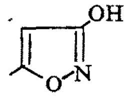
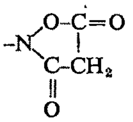
§Taken from earlier studies

Table 2 ω-Acidic terminals of excitatory amino acids

General structure:



$$\begin{matrix} H_2N \\ HO_2C \end{matrix} > CH\text{–}CH_2\text{–}R$$

R	Compound	Reference
$-CH_2-CO_2H$	Glutamic acid	9, 11
 $-C(=CH_2)-CO_2H$	4-Methylene glutamic acid	12
 $-CH(F)-CO_2H$	4-Fluoroglutamic acid	12
$-CH_2-SO_3H$	Homocysteic acid	13
$-S-SO_3H$	S-Sulphocysteine	*
$-CH_2-SO_2SH$	2-Amino-4-thiosulphonylbutyric acid	†
$-NH-CO-CO_2H$	β-N-Oxalyl-α,β-diaminoproprionic acid	14
	6-Hydroxy-2-pyridylalanine	†
	2,4,5-Trihydroxyphenylalanine (6-Hydroxy-dopa)	†
	Ibotenic acid	15
	Quisqualic acid	This paper

*D. R. Curtis, D. Felix and J.C.W. (unpublished).
†R.H.E. and J.C.W. (unpublished).

excitatory transmitter remains uncertain. Clearly, the need for specific antagonists of synaptic and chemical excitation grows increasingly more important.

We thank Professor T. Takemoto (Tohoku University) for gifts of domoic, quisqualic and α-allo-kainic acids. Support from the MRC and the National Fund for Research into Crippling Diseases is acknowledged.

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Prolactin-stimulated production of somatomedin by rat liver

GROWTH hormone (GH) and pituitary prolactin (PP) are structurally homologous and have functional similarities both in the lower vertebrates[1] and in mammals[2]. In the whole animal a major function of GH is to stimulate the longitudinal growth of the skeleton, and GH acts by controlling the production of a second series of hormones, the somatomedins. The somatomedins are present in serum and will stimulate the rates of division and matrix production of chondrocytes *in vitro*[3,4]. GH itself has no effect *in vitro* on the cartilage cells, the chondrocytes.

It is now known that GH will stimulate both liver[5] and kidney[6] to produce the somatomedins and that in man the liver is a major site of somatomedin production[7]. If bovine growth hormone (BGH) in a chemically defined medium is perfused through rat liver, somatomedin activity is subsequently found in the perfusate; this somatomedin(s) has properties similar, in many respects, to that found in serum[8–10]. Pharmacological doses of BGH (1–10 μg ml^{-1}) have to be used for significant somatomedin activity to be detected in the perfusates At the concentrations of BGH used, more physiological levels of bovine PP (10–100 ng ml^{-1}) would have been present. We have therefore measured the stimulation of somatomedin activity by PP at concentrations up to 500 ng ml^{-1} in the perfused rat liver system. Our results demonstrate that in this system PP stimulates the production of somatomedin with properties similar to that found following GH perfusion.

Livers from normal male Sprague–Dawley rats (220–260 g) were perfused as described by Dehnel[8]. The liver was perfused first with 100 ml Waymouth's medium[11] supplemented with 0.5% (w/v) bovine serum albumin (Cohn fraction V) to wash out endogenous somatomedin activity, the final 15 ml being collected as a liver control. A further 100 ml Waymouth's medium, containing 0.5% bovine serum albumin, was then circulated three times through the same liver; this medium contained either no added hormones (control perfusion) or BGH (0–10 μg ml^{-1}) or OPP (0–500 ng ml^{-1}). Following perfusion red cells were separated by centrifugation and somatomedin activity assayed by Dehnel's modification of the technique described by McConaghey[5].

Ovine pituitary prolactin (OPP) from both sources at concentrations of 0–500 ng ml^{-1} had no significant effects on costal cartilage metabolism as measured by the rate of $^{35}SO_4$ uptake, an index of cartilage proteoglycan biosynthesis (Table 1). After perfusion through rat liver, media containing either OPP (50 ng ml^{-1}) or BGH (1 μg ml^{-1}) now significantly ($P<0\ 001$) stimulated $^{35}SO_4$ uptake whereas media after control perfusions did not (Table 1). The optimal increase in $^{35}SO_4$ uptake (Table 1) was present after perfusion with either OPP (50 ng ml^{-1}) or BGH (1.0 μg ml^{-1}). All these perfusions were carried out with livers of animals killed at 1100. In contrast perfusion with OPP (50 ng ml^{-1}) of livers of animals killed at 1500 only stimulated $^{35}SO_4$ uptake marginally. Perfusions with BGH clearly stimulated $^{35}SO_4$ uptake at both times (Table 1). Circadian responses to prolactin have been described for other prolactin-stimulated systems[14,15].

Preliminary studies on the properties of the somatomedin activity produced after perfusion of rat liver with OPP demonstrate that they are similar to those we have shown

Table 1 Stimulation of $^{35}\mathrm{SO}_4$ uptake by costal cartilage by media containing PP, or GH, before and after perfusion through rat liver

	Uptake (c.p.m. %) compared with basal media control (hormone added at assay stage)	No. of perfusions	Uptake (c.p.m. %) compared with liver control (hormone added at perfusion stage)
Controls			
Liver control	121±22 (75)	—	100±15 (75)
Waymouth's medium	100± 7(102)	—	82+14(102)
Ovine prolactin			
0 ng ml^{-1}	—	3	113±18 (9)
5 ng ml^{-1}	96± 8 (8)	3	138±28 (9)
50 ng ml^{-1}	99±10 (11)	6	187±15 (15)*
50 ng ml^{-1}(NIH)	106± 7 (7)	6	266±53 (17)*
50 ng ml^{-1}(1500)	—	6	119± 8 (18)†
500 ng ml^{-1}	83± 8 (9)	5	171±14 (15)*
Bovine growth hormone (NIH)			
100 ng ml^{-1}	103±10 (6)	2	77±16 (4)
1 μg ml^{-1}	95±12 (6)	3	181±24 (9)*
10 μg ml^{-1}	121± 6 (5)	2	164±16 (5)*
10 μg ml^{-1} (1500)	—	2	192±27 (6)*

For each assay, costal cartilage sections (3–4 mm long) were taken from each of five Sprague–Dawley rats, each of 50–60 g; the costochondral junctions were discarded. Five cartilage sections, one from each rat, were added to 2.0 ml of the sample to be assayed and the reaction started by the addition of 1.0 μCi ml^{-1} $^{35}\mathrm{SO}_4$ (carrier-free from Radiochemical Centre, Amersham). Each sample was assayed in triplicate. Flasks were maintained at 38 °C for 48 h in a shaking water bath, with an atmosphere of 5% CO_2 in air The reaction was stopped by immersion in boiling water and the uptake of $^{35}\mathrm{SO}_4$ into cartilage proteoglycans estimated by liquid scintillation counting according to the method of McConaghey[5]. Results are expressed as c.p.m. $^{35}\mathrm{SO}_4$ uptake per mg wet weight cartilage compared to the liver control (see text). Each figure is the mean ±s.e. for the number of assays given within the brackets. Unless otherwise stated all perfusions were carried out at 1100, using ovine prolactin (Sigma).

Significance of differences between means assessed by Student's t test: $*P<0.001$; $\dagger P = 0.05$–0 02. The difference between $^{35}\mathrm{SO}_4$ uptake after incubation in medium +50 ng OPP/ml perfused at 1100 and the same medium perfused at 1500 is significant at $P<0.001$.

BGH used was batch B17 from NIH (0.92 IU GH mg^{-1} and <0.50 IU PP mg^{-1}), (ref. 12 and personal communication). OPP preparations were obtained from NIH batch S-10 (26 IU PP mg^{-1} and <0.01 IU GH mg^{-1}) (ref. 12 and personal communication) and from Sigma, batch L-4876 (about 30 IU PP mg^{-1}). Acrylamide gel electrophoresis[13] of these preparations demonstrated that there was less than 5% contamination of the BGH with bovine PP and of the OPP preparations with ovine GH.

after BGH perfusion[8-10]. Thus activity cannot be replaced by the addition of serine and glutamine, which both stimulate cartilage metabolism[16] and which are the only two amino acids present in suboptimal concentrations[9] in Waymouth's medium (Table 2). In addition the OPP-induced somatomedin activity, like that induced by BGH, will stimulate $^{35}\mathrm{SO}_4$ uptake by isolated rabbit chondrocytes in the assay of Ash and Francis[10].

These results demonstrate that both preparations of OPP stimulate somatomedin production by rat liver in the *in vitro* conditions used in the present work. Could these effects of OPP be explained by contamination with ovine GH? The preparations of OPP used contain low levels of GH and are more potent than the BGH used (Table 1). Although we have so far been unable to test ovine GH directly in the liver perfusion system, it is known that ovine GH and BGH have similar potencies in both the weight-gain test in 100 g hypophysectomised rats[12] and the rat-tibial-width test[17]. It is thus most unlikely that the effects of OPP on liver somatomedin production are caused by contamination with ovine GH as the latter would have to be many times more potent than BGH. The distinct actions of BGH and OPP when perfused through the rat liver at different times of the day also provide evidence that each can act separately on liver to stimulate somatomedin production.

Recent clinical evidence has suggested that PP can stimulate *in vivo* both somatomedin production (detectable in serum) and continued skeletal growth in the absence of immunologically detectable GH (refs 18 and 19). These results, together with those presented here, demonstrate that pituitary prolactin can act to stimulate somatomedin production and thus function as a skeletal growth hormone. Further work will be required to elucidate the relative importance of prolactin and growth hormone in the control of mammalian skeletal growth in normal physiological conditions.

Table 2 Properties of somatomedin activity produced following perfusion of rat liver with OPP (NIH)

	No. of perfusions	Uptake (c.p.m %) compared with basal medium
Waymouth's medium	—	100±18(14)
OPP perfusion medium	3	166±21(9)*
Waymouth's medium+serine+glutamine	—	136±13(8)
OPP perfusion medium+serine+glutamine	3	211±24(7)*

Assays were carried out as in Table 1. Serine and glutamine were added where indicated to bring the final concentrations in the assay medium to 1.75 mM l^{-1} and 3.5 mM l^{-1}, respectively[9]. Perfusions were carried out with OPP (NIH; 50 ng ml^{-1}).

$*P$ against controls <0.02.

This work was supported by a grant from the MRC to Professor R. B. Duthie. BGH was a gift from Dr A. E. Wilhelmi (NIH) and OPP from Dr D. Horrobin. We thank Dr L. E. Reichert, Jr for information on the potencies of the NIH hormones.

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Received December 23, 1974, accepted April 1, 1975.

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matters arising

Feeding analogues

THE recent letter[1] on the control of food intake recalls the old caveat that it is very easy to make an analogue; the problem is to know what it is an analogue of.

The analogue presented invokes operation of an on–off feeding switch by the level of 'energy flow'. It is not at all clear what flow of energy is meant. The values used for turn-on and turn-off of feeding roughly correspond with those estimated by Le Magnen and Devos[2] as 'food utilisation', meaning rate of absorption from the gut. The block diagram, however, shows the effective energy flow after the summing point with lipogenesis–lipolysis; that is, for practical purposes, the energy flow triggering the feeding switch of the analogue is total metabolic rate. Now, for rats, the mean increment in metabolic rate from the beginning to the end of a meal (or, with sign change, from the end of one meal to the beginning of the next) is not more than 10%, and is often much less or even negative[2,3]. It does not approach the 230% (18–60 calorie min^{-1}) proposed by Toates and Booth[1]. In the case where the beginning of a meal coincides with the change from rest to activity, and general motor activity continues beyond the end of the meal, then increase in metabolic rate from just before the onset of the meal to its termination can be about 75%, but that change is almost entirely a function of the change from rest to activity with only a small contribution from feeding as such[3].

We therefore seem to have the situation in which energy flow is interpreted as rate of energy absorption from the gut but is inserted into the analogue as if it were metabolic rate; or in which it is interpreted as metabolic rate but gut absorption values are mistakenly used. This is not just a quantitative discrepancy. Even if 'energy flow' denotes metabolic rate and even if realistic values for metabolic rate are used the analogue as presented would have a nonsense output. Since the major changes in metabolic rate are attributable to change between rest and activity and since feeding must be a part of activity[3], feeding by this analogue can only occur during rest, when, by definition, it cannot occur.

The approximate agreement shown between real rats and the analogue for some variables could indicate that these variables are too insensitive for assessment of the function of the model.

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[1] Toates, F. M, and Booth, D. A., *Nature*, 251, 710–711 (1974).

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TOATES AND BOOTH REPLY—We stated in our second paragraph[1] that the energy flow controlling feeding in our model is that sampled by hypothetical receptors in neural tissue. As the text and figure specify, we make a crude estimate of this by summating the large and considerably variable flow of energy from the gut, with the circadian variations in net flow from or to triglyceride. We are concerned with some of the energy flows between tissues, not the energy flows between the organism and the environment. The dilemma Morrison[2] poses is therefore fallacious. In a later version of the model[3] we allow for circadian variations in heat production by non-receptor tissues; metabolic rate data assist us there, although (as Morrison says[2]) such variations are relatively small. The physiological variables we use are sufficiently sensitive to give unrealistic behavioural predictions when set at values outside the observed range.

We heartily concur with Morrison's point[2] that the empirical interpretation of mathematical models is a treacherous business. We have been able to discuss the relationships between this model and the facts in more detail elsewhere[3]. We welcome this opportunity to counteract misunderstanding that a necessarily brief report almost inevitably permits.

[1] Toates, F. M, and Booth, D. A, *Nature*, 251, 710–711 (1974).

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Lead in children's teeth

DAY, HART AND ROBINSON[1] examined the geographical dependence of lead concentrations in urban street dust in several ways. When subdividing data according to city quadrant they noted that the four values ranged from 91 to 122% of the mean value of 970 p.p.m.

We divided our data geographically when studying lead concentrations in crowns of permanent premolar teeth collected from Bristol school dental clinics. The data, plotted according to the home location of the children, fell equally on both sides of a line passing through the centre of the urban area and the Avonmouth industrial complex, approximately 10 km to the north-west. This orientation, however, is approximately at right angles to lead and zinc concentration contours[2] based on extensive sampling of soil and of elm and hawthorn leaves in connection with the regional environmental survey (Sabrina Project).

Table 1 Lead concentrations in tooth crowns according to city zones

Zone	Mean concentrations (p.p.m.)			
	Pb	Zn	Cd	Cu
NE	71±19*	132±32*	4.3	6.6
SW	53±16*	143±36*	4.4	6.6

* Standard deviations (28 samples). Difference (north-east/south-west) significant for Pb ($t = 3.7$, $P<0.001$).

Concentrations of lead in Bristol children's teeth are typically 60 p.p.m. A mean value for tooth samples from 28 children living in a zone to the south-west of the chosen demarcation line was only 75% of that for the same number representing the zone to the north-east (Table 1). There was a non-significant difference in the opposite direction for zinc analyses; the values for cadmium and copper were the same in the two zones.

Another aspect of this type of environmental observation—but in which no geographical distinction was evident—is provided by a study[3] of concentrations of lead and other metals in developing teeth in cases of stillbirth and neonatal death. There was significantly more lead in the dentitions of those born in 1972–73 than in those dating from 1957–63.

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Factors influencing effect of hydrocortisone on rat brain tryptophan metabolism

THE concentrations of rat brain 5-hydroxytryptamine (5–HT) and its metabolite 5-hydroxyindole acetic acid (5–HIAA) have been found to be decreased by injection of hydrocortisone[1–3] or other corticosteroids[4]. Benkert and Matussek[5] did not, however, observe changes in 5–HT concentration at various times after injecting hydrocortisone acetate. More recently, Hillier *et al.*[6] reported no change of 5–HT but a rise of 5–HIAA 3 h after hydrocortisone (15 mg kg^{-1} intraperitoneally). The latter authors also found no change in serum total tryptophan and an increase in free serum tryptophan at this time. The following observations may clarify these contradictory findings.

Table 1 Effect of hydrocortisone on tryptophan metabolism in rats

Injected	Plasma tryptophan (μg ml^{-1})		Tissue tryptophan (μg g^{-1})		Brain 5–hydroxyindoles (μg g^{-1} wet weight)	
	Total	Free	Liver	Brain	5–HT	5–HIAA
Saline	27.04±2.05	3.92±0.84	4.89±0.51	2.04±0.19	0.45±0.05	0.87±0.12
Hydrocortisone	22 77±3.38*	1 84±0.51‡	3.66±0.35†	1.45±0.09‡	0.38±0.05	0.51±0.06‡

Male Sprague–Dawley rats (Anglia Laboratory Animals, Alconbury) 35 d old were used. Animals were killed 6 h after injection. Plasma tryptophan was measured as described previously[10], tissue tryptophan by the method of Denckla and Dewey[11] and brain 5–HT and 5–HIAA by the method of Curzon and Green[12]. Results expressed as mean ± s.d. of six observations. Different from saline injected controls.
* $P<0.05$.
† $P<0.01$.
‡ $P<0.001$.

Table 2 Influence of age on effect of hydrocortisone on kynurenine production by isolated perfused rat liver

Age (d)	Injected	Hepatic kynurenine production (μg^{-1}g^{-1}h^{-1})
35	Saline	35±5(4)
	Hydrocortisone	139±32(4)*
100	Saline	22±8(4)†
	Hydrocortisone	65±31(5)*‡

Livers were perfused by the method of Hems *et al.*[13] with a semi-synthetic medium[14] containing tryptophan (1.0 mM). Rats were injected with hydrocortisone (5 mg kg^{-1}) or saline 3 h before the start of the liver perfusion. Results expressed as mean ±1 s.d. with number of observations in brackets.
* Different from saline-injected control rats of same age $P<0.01$.
† Different from saline-injected 35-d rats $P<0.05$.
‡ Different from hydrocortisone-injected 35 d rats $P<0.01$.

- Using the hydrocortisone dose of Hillier *et al.*[6] of 15 mg kg^{-1}, we confirm that there is no change in total plasma tryptophan 3 h after injection of the steroid, but also found no alteration in free tryptophan. In the original work however[1,2] 5 mg kg^{-1} hydrocortisone (as the sodium succinate derivative) was injected intraperitoneally and brain 5–HT and 5–HIAA determinations were made 6 h later, at which time the decrease in both compounds was maximal. Using this protocol we now find a fall of both total and free plasma tryptophan and a fall of tryptophan concentration in both liver and brain (Table 1). Brain 5–HT decreased but not significantly, while 5–HIAA was considerably and significantly lowered (Table 1).
- Brain tryptophan, 5–HT and 5–HIAA are all unaltered following hydrocortisone (5 mg kg^{-1}) administration to gerbils[7]. In these animals (unlike the rat) the hepatic enzyme, tryptophan pyrrolase is not induced by hydrocortisone[7,8]. This is consistent with an association between the increased hepatic pyrrolase activity in rats following hydrocortisone[2] and the changes in tryptophan metabolism now reported.
- Other factors may also be important when studying the effects of hydrocortisone on tryptophan metabolism. The time courses of hydrocortisone actions depend on the form in which it is injected. Hydrocortisone sodium succinate leads to more rapid changes of pyrrolase activity than the less water soluble hydrocortisone acetate or free steroid[9].
- The age of the animals is also important. Older animals (100 days or more) do not exhibit the brain 5-hydroxyindole changes or the large hepatic pyrrolase induction shown by younger rats (35 days; 120 g) following the same hydrocortisone dose per kg body weight[9]. This smaller increase of pyrrolase activity in older animals was previously demonstrated by measuring enzyme activity in liver homogenates after hydrocortisone treatment, and has now been demonstrated in the perfused liver. Thus, kynurenine production from tryptophan by isolated perfused rat liver preparations is much lower in older animals both before and after hydrocortisone (5 mg kg^{-1}) pretreatment 3 h previously (Table 2).

Thus we have shown that hydrocortisone can alter tryptophan metabolism both peripherally and in the brain and confirm that changes are consistent with decreased brain 5–HT synthesis. These changes are, however, dependent on several factors; times of observation, age of animals and the hydrocortisone preparation used. Though it would be rash to extrapolate too readily from acute pharmacological experiments to a chronic illness such as endogenous depression, the possibility exists that similar changes have significant roles in the aetiology of psychiatric disorders in some subjects[1,2] or, indeed, in the 5–HT metabolism of normal subjects in certain circumstances.

This work was supported by the Medical Research Council We thank Miss J. P. Hughes and Mr. P. Hutson for technical assistance.

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Narrow-angle cosmic-ray anisotropies

SPELLER, *et al.*[1] have presented a measurement of the diurnal cosmic-ray anisotropy, as found with underground muons in London. An additional, but quite different, interpretation of their data is, however, possible.

From the widely accepted diffusion models for cosmic-ray propagation in the Galaxy, one could expect only a diurnal anisotropy (24-h wave). A newer model[2], based on adiabatic propagation of cosmic rays, predicts that an excess could occur within some small region of the celestial sphere. The direction of the excess would depend on the orientation (in space and time) of the source object relative to galactic magnetic field lines which connect the source with the Solar System.

A narrow-angle anisotropy near the celestial equator was reported[3] and confirmed[4] during the time of the London experiment (see Table 1). It resembled an earlier anisotropy[5] in that it was observed at the time of a sunspot minimum, then gradually faded away. The London data are shown in Fig. 1, split into periods before, during and after the 1964 anisotropy. The point at 18 h is $3.2\sigma_N$ (standard deviations) above the curve.

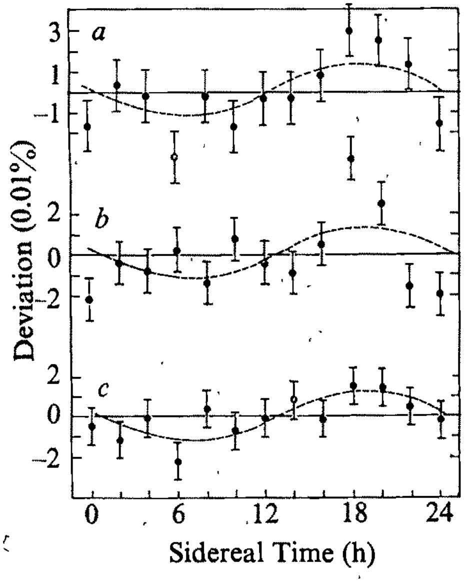

Fig. 1 The London data for: *a*, 1961–62; *b*, July 1963–June 1964; *c*, 1965–68, showing the deviation from the average intensity against sidereal time. The sine curve fit to the total data is shown. The anisotropy is present in *b*, but lacking in *a* and *c*, in agreement with ref. 3.

The apparent position of this peak agrees with the Winnipeg data[3] if one includes geomagnetic deflections calculated for particles of 100–200 GeV. (This explanation is inadequate for the reported Hong Kong position[4]. Other factors, such as accessibility to lower declinations, may have played a role.) This estimated energy is above the thresholds of the Winnepeg and Hong Kong detectors but is near the London threshold at a zenith angle of 51° (the efficiency is about 10% at 160 GeV and 90% at 1600 GeV). The small size of the anisotropy in the London data (0.03%, by comparison with 0.3% at Hong Kong and 10% at Winnipeg) can be understood in terms of the energy threshold, the large zenith angle and the wide angular aperture.

Table 1 Narrow-angle anisotropies

Experiment location	Nagoya	Winnipeg	Hong Kong	London
Latitude (°N)	35	50	22	51
Period observed	1952–56	Dec. 1963–Sept. 1964	Jan. 1964–Mar. 1964	1963–64
Not observed	1957–61	Nov. 1964–Dec. 1964	—	1965–68
Declination (°N)	0.5	0±18	—	—
Right ascension (h)	5.25	20.5*	23*	19*
Angular aperture (°)	5×7	±18	±45	±63
Particle rigidity (GV)	280±40	>40	>30	150±50
Significance (standard deviations exceeded)	6	16	5	5

*Uncorrected for geomagnetic deflection.

The uncertainties σ_N shown in Fig. 1 arise from counting statistics only. The London authors argue for the use of $1.4\sigma_N$, but the scatter of their data around their sine curve in Figs 1*a* and *c* is $\sigma_S/\sigma_N = 0.89$ and 0.84, whereas the expected scatter is $\sigma_S/\sigma_N = 0.93 \pm 0.20$. If one removes the two points at 18 h and 20 h, the other points fit well to a straight line. The two points are $5.0\sigma_N$ and $2.9\sigma_N$ above this line.

During previous sunspot minima, conditions have apparently become favourable enough to observe two narrow-angle anisotropies of rather low energy. Each would be caused by a cosmic-ray source near our magnetic field line in the Galaxy. The next sunspot minimum is expected early in 1976 (ref. 6).

I thank H. E. Bergeson for discussions and the London authors for correspondence and data. This research has been supported by NASA.

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ELLIOT AND THAMBYAHPILLAI REPLY—We are sceptical about Barrowes's interpretation of our (London) data because: (1) We disagree with the use of $\sigma_N = 100/\sqrt{N}$ % in assessing the statistical reality of the deviation from the mean at 18 h. Our own analysis of the scatter of harmonic coefficients shows that a value of $\sigma_N = 1.8\times(100/\sqrt{N})$ % is more appropriate than the lower limit used by Barrowes.

(2) The energy thresholds given in his Table 1 do not in themselves provide a satisfactory basis for comparing the magnitude of the effect to be expected at the various stations. To do this, it is necessary to use the complete response functions for the detectors concerned.

(3) We cannot see how a narrow-angle anisotropy can be observed at the Earth for primary cosmic rays with $E \leqslant 200$ GeV because of the wide spread in the deviation of such particles in the interplanetary magnetic field. The angles through which these particles would have been deflected by the field at any time during the recent solar cycle is much greater than that in the geomagnetic field.

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Messenger RNA abundance and gene regulation in eukaryotes

BISHOP *et al.*[1] found three abundance classes for poly(A)-containing mRNA in HeLa cells (17 sequences of about 8,000 copies per cell, 350 of about 440 copies and 35,000 sequences of about 8 copies). This situation may be quite general. Green, Goldberg and Todaro[2] found three ranges of relative collagen synthesis rates (1.5–14%; 0.15–0.4% and beyond 0.002%) and even where only one or two abundance classes are found, non-poly(A)-containing mRNA may contain another abundance class[3].

A rather simple control system for gene regulation, similar to one previously proposed[4,5], may explain this rather reduced number of mRNA abundance classes which should correspond to three possible degrees of gene activation. Histones usually block DNA transcription, but specific activators (probably non-histone nuclear proteins) controlled by some interrelated gene triggers, may open different DNA portions to transcription by combining with "promoter" regions. This would be the first control level. Within the unblocked DNA portions a second control level could act, based on less stringent repressors (of the type found in bacteria) also controlled by triggers of interrelated genes. High abundance mRNA should correspond to unblocked–derepressed structural genes, medium abundance mRNA to unblocked–repressed structural genes. The many very low abundance mRNA sequences may correspond to accidental transcription of blocked structural genes during DNA replication, which may also be required for unblocking DNA portions in cell differentiation ("quantal mitosis"[6]). Alternatively, "switching on" of histone acetylation and phosphorylation may produce a general weakening of the histone block on transcription, allowing the appearance of the very low abundance mRNA class.

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Structure of graphite

ERGUN[1] has suggested a structure for graphite based on new X-ray diffraction data, in which two of the carbon–carbon bonds lengths in the six-membered ring of any layer are shorter than the other four. This gives rise to the so-called 1,4 quinoid structure. The atomic positions in this structure are

$$(0,0,0), (x,\bar{x},0), (\tfrac{1}{2}+\tfrac{x}{2}, \tfrac{1}{2}-\tfrac{x}{2}, \tfrac{1}{2}),$$
$$(\tfrac{1}{2}-\tfrac{x}{2}, \tfrac{1}{2}+\tfrac{x}{2}, \tfrac{1}{2})$$

with $x = 0.318$. The resulting square of the geometric structure factor is then[1]:

$$F^2 = 16\cos^2 \pi x(h-k)\cos^2(\pi/2)\times$$
$$\times\ [(1-x)(h-k)+l]$$

(If the value of x is $\frac{1}{3}$, then the commonly accepted graphite structure results. That structure comprises regular hexagonal nets, stacked in the sequence . . .[AB] . . .) Ergun used his measured values of the four observed ($h\ h\ 0$) reflections (the F^2 values of which, according to the equation shown here, are independent of the parameter x) to obtain a function that he did not publish but which he used to reduce the other seven observed ($h\ k\ 0$) intensities to an F^2 basis. The value for x of 0.318, which was apparently obtained by trial and error, was then used to calculate values for the remaining seven $F^2{}_{hk0}$ values. The agreement between $F^2{}_{\mathrm{obs}}$ and $F^2{}_{\mathrm{calc}}$ is astonishingly good, with

$$R_2 = 100x\Sigma|F^2{}_{\mathrm{obs}}-F^2{}_{\mathrm{calc}}|/\Sigma F^2{}_{\mathrm{obs}}$$
$$= 1.25\%$$

Ergun stated that "it was evident [on the basis of his observation of sharp diffraction maxima] that the lattice is hexagonal", by which he meant $a = b$ with an angle of 120° between them. The symmetry of his structure is, however, not hexagonal, but orthorhombic. Thus, not all reflections having identical spacings have the same calculated values of F^2. In particular, reflections having the same value of (h^2+hk+k^2) will occur at identical scattering angles, but not all of these are symmetrically equivalent.

For example, the reflection (210), with $F^2{}_{\mathrm{calc}} = 1.075$, $F^2{}_{\mathrm{obs}} = 1.08$ (Ergun's figures) occurs at the same diffraction angle as $(3\bar{2}0)$ and $(3\bar{1}0)$, and the calculated F^2 values of these two reflections, as given by the equation already shown, are 0.449 and 1.184. The average relative value of $F^2{}_{\mathrm{calc}}$ is thus 0.903. The adjacent reflection, (200), with $F^2{}_{\mathrm{obs}}= 0.80$, is, in Ergun's table, in excellent agreement with $F^2{}_{\mathrm{calc}}= 0.804$. This reflection is actually, however, the weighted average of (200) and $(2\bar{2}0)$, and the latter, with $F^2{}_{\mathrm{calc}}=1.184$ leads to a relative $F^2{}_{\mathrm{calc}}$ of 0.931. The calculated F^2 ratio for (200)/(210) is thus 1.031 instead of the 0.748 given by Ergun, and is no longer in almost exact agreement with the observed ratio of 0.741.

Because Ergun did not report his raw intensity data, but only those "corrected" with an unknown function it is not possible to use them to come to any further conclusions regarding the structure of graphite. But in any case it is clear that no support for the quinoid structure is provided by the spectacular agreement between the values of $F^2{}_{\mathrm{calc}}$ and the $F^2{}_{\mathrm{obs}}$, which now becomes merely interesting.

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Environmental modification of a genetically based behaviour pattern in *D. melanogaster*

THRELKELD *et al.*[1] have shown that the reduced mating success of mutant yellow males with normal females of *Drosophila melanogaster*[2] can be alleviated by genetic selection. It is important to realise that genetically based differences in behaviour are not rigid but are contingent on the environment in which they are measured.

In the present study to determine the environmental modifications necessary to increase the mating success of yellow males, two inbred strains of *D. melanogaster* were used, one wild type and the other yellow. All matings were conducted at 25 °C under continuous light and were always between virgin 1–2-d-old wild-type females and yellow males. Each pair of flies was anaesthetised with ether, placed in a vial (23 × 85 mm) containing 10 ml of yeasted propionic acid medium and left for 9 d. A mating was scored as successful if progeny were present at the end of this time.

Table 1 Percentage mating success

Sex ratio	Sample size	Matıng success
1:1	249	0.8
1:2	64	1.6
1:3	37	21.6
1:4	78	21.8
1:5	53	9.4

The sex ratio was varied from 1 : 1 to 1 : 5 (female : male). The results (Table 1) reveal significant differences between the 1 : 1 ratio and both the 1 : 3 ($P<0.001$) and the 1 : 4 ($P<0.001$) ratios. These remain significant even if mating successes are compared on a per male basis. It would seem that mating success is not a simple linear function of the number of males present and that a highly favourable interaction effect occurs when the ratio of males to females is three or four to one.

Artificial selection for seven generations was able to increase the competitive mating success of yellow males from 36% to 67% (ref 1). A change in sex ratio can increase non-competitive mating success from less than 1% to 22%. Environmental modification can produce significant changes in genetically based behaviour patterns.

This work was supported by a Scholarship from the National Research Council of Canada.

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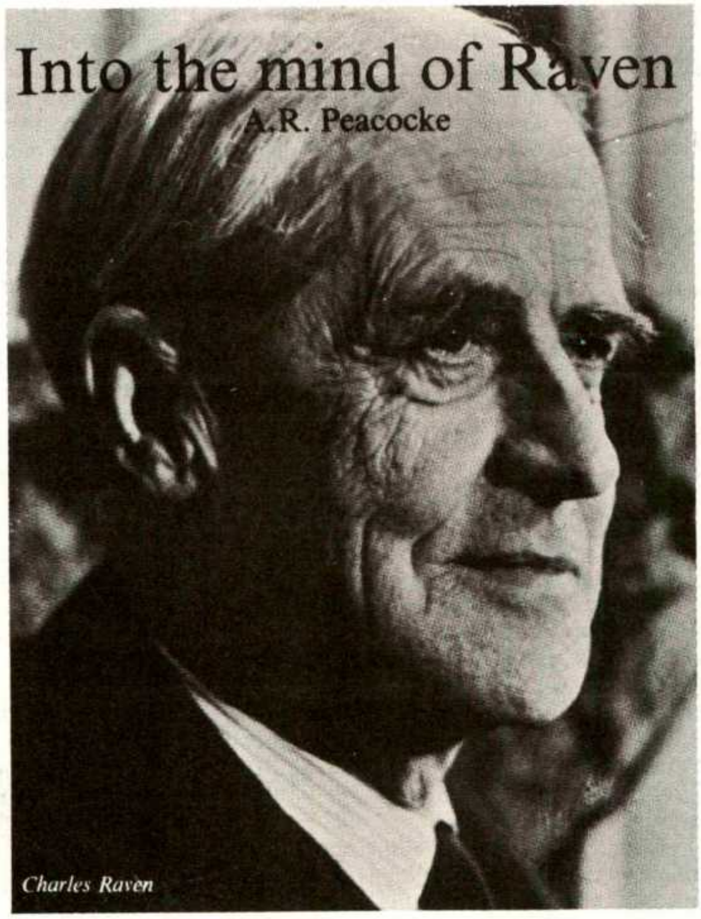
Into the mind of Raven

A.R. Peacocke

THE biography* of a leading theologian would not ordinarily find reviewing space in the columns of *Nature*. But there was nothing ordinary about Charles Raven either as a theologian or as a man. What other Regius Professor of Divinity at Cambridge (or anywhere else) has, while in office exercising his normal academic duties, produced a monumental biography of a man of science? The man of science was, of course, John Ray, the first and perhaps the greatest, British naturalist, who produced the first accounts of county flora in 1660 and a comprehensive *History of Plants*. If for nothing else, we owe Charles Raven's own biographer, Dr F. W. Dillistone, an immense debt for reminding us of the devoted and meticulous research with which Raven brought his task to its accomplishment when he published *John Ray: Naturalist* in 1942. Raven, so Dillistone tells us, collated every record of Ray's travels and research, translated countless descriptions and identifications from Latin and Greek, and collected nearly all the plants, birds and insects that Ray records—often from the same localities.


Charles Raven

The resulting classic biography was itself the work of a unique man and Dr Dillistone's immensely readable, sensitive and thoughtful account of Dr Raven's life and work takes the veil off Raven's mind, taking us as far as we are ever likely to be able to penetrate into that vast treasure house. Dillistone shows us how Raven's bond of sympathy with Ray lay in their both sharing a vision of the unity of scientific and religious experience, that vision partly expressed in the title of Ray's widely influential *The Wisdom of God in the Works of Creation* (1691).

Raven was a born naturalist—observant, sharp-eyed, patient (able to wait for long hours in atrocious weather for his observations)—a beautiful illustrator and draughtsman (learnt from his mother), an accomplished Latinist (undaunted, indeed revelling in, the Linnean scheme) and an avid collector, with an accurate and comprehensive memory. Dillistone shows how the zest for living creatures never left Raven and how his joy and thrill in the careful observation of nature was fundamental to his total vision. He takes us through Raven's formative years at home and at the University of Cambridge and makes it clear to those of us who knew Raven only at a distance how it was that a student of first the classics and then divinity could have had such a zest for and sympathy with the natural sciences. Apparently this zest and sympathy originated from his mother's training, so that perhaps we are less surprised when Dillistone tells that while working for the Divinity Tripos, Raven attended the Lectures of William Bateson, who was one of the outstanding exponents, with a deterministic emphasis, of the new genetics during the early years of this century. This, so Dillistone judges, and those of us who remember Raven recalling his experience of Bateson's lectures will agree, was one of the chief goads to Raven's intellectual quest through his life—the interpretation of evolution as a non-deterministic and creative process which is the clue to the natural emergence of the human person. The consummation of this process Raven saw in a transmutation of human personality by the illumination and extension of human existence, which the Christian faith provided through its focus on the man in history in whom it was first achieved and who thereby opened the way for others.

By enriching this biography with a careful assessment of the context of thought within which Raven moved at various stages of his life, Dr Dillistone has provided us not only with an account of an exceptionally gifted man, but also with a chronicle and diagnosis of the ebb and flow of that Christian liberalism of which Raven, along with William Temple, was one of the most brilliant exponents. For this reason, this biography comes at an especially opportune time, when the near-collapse of radical politicised versions of Christianity, at least among students in this country, has left the enquiring with apparently only one other alternative—that of a conservative biblicism which Raven regarded as an affront to the intellectual integrity of any critically enquiring youthful mind ready to apply the same standards of judgment to its religion as to other intellectual enquiries, including that of science.

With Raven lecturing, writing and preaching in Cambridge, a critical, enquiring, explorating, yet at the same time, integrating and visionary Christian outlook was clearly available as an alternative to such conservatism, and Dillistone reminds us how science and mathematics students responded to it; men whose subsequent careers were

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**Charles Raven: Naturalist, Historian, Theologian.* By F. W. Dillistone. Pp. 438+18 photographs. (Hodder and Stoughton: London, 1975.) £5.25.

as different as those of Charles Coulson (the theoretical chemist who became Rouse Ball Professor of Applied Mathematics at Oxford) and Ian Ramsey (who became Nolloth Professor of the Philosophy of Religion at Oxford and later Bishop of Durham). Dr Dillistone's biography is opportune because the conservative, intellectually uncritical kind of Christianity from which Raven released so many, is today attractive in its simplistic unambiguity to many of the young who can scarcely hear any other voice. "Raven, thou shouldst be living at this hour", but, failing that, we can again catch those rich and profound cadences through his faithful biographer.

But Dr Dillistone is no adulatory sycophant. He shows us where this idol had feet of clay: his failure to understand the crises of continental thought under the impact of the Nazis; his over-magisterial non-suffering impatience with 'fools'; his too-rapid dismissal of any dialectical, non-unified system of thought; his almost pathological concern with his supposed rejection by "the Establishment" of his day on account (he supposed) of his unpopular pacifist views and of his support for the ordination of women.

More positively, Dr Dillistone has dug deeply to discover accounts of Raven's moments of illumination which carried him through the devastating experiences at the Front in the first World War and through tragic bereavements at critical moments in his life. The inner life of the Regius Professor, the Master of a Cambridge college and the Vice-Chancellor of that university, are gently, surely and shrewdly unveiled for us in a way which shows how what Raven loved to call "the sublime law of sacrifice" (quoting Fabre) lay behind the public image.

Those of us who have known Raven through his writings can, with the help of Dr Dillistone, now meet him as the complex, exceptionally gifted and contradictory man he was. Even more important, those who have known neither him or his writings can, through this sympathetic biography, now be inducted into the thought of a man who combined enthusiasm for science with insight into religious experience into a unique amalgam so clearly needed by our disrupted and fissiparous culture. For Raven, in both spheres, the exploration was liberal and open-ended and productive of that unity of vision shared, across the centuries, with Ray.

Not whole but just some of the parts

Microbial Communities in a Forest-Rendzina Ecosystem: The Pattern of Microbial Communities. By I. M. Szabo. Pp. 415. (Akademiai Kiado; Budapest, 1974.) £10.50.

THE microbial ecology of soil is a subject which has in the last few years acquired a voluminous literature at an ever increasing rate. Unfortunately, this has been achieved without any comparable increase in the understanding of the systems under study. The diversity of microbial communities, their functions and their microhabitats have obscured all attempts to derive any but the most basic ecological generalisations. Professor Szabo attributes this failure, in part, to a dispersal and dilution of research effort. In spite of the complex nature of the system (or perhaps because of it) research planning has tended to concentrate on specific functions, organism groups, or ecological parameters. This has resulted in a science in which there is considerable knowledge of some of the parts but little understanding of the whole.

The philosophy of the studies reported in this volume is different. For over 15 years Professor Szabo and his colleagues have conducted an intensive study of soil microflora and fauna in a single area of oak-hornbeam forest in western Hungary. The concentration on a single site is deliberate. Only in that way can "those general principles which govern the activities of complex saprophytic communities in the soil" be recognised and understood. It is in that context that the reader is asked to judge the contents of this book.

The first chapter describes features of the forest site at the ecosystem level; in Chapter Two the focus becomes finer and we are given accounts of micromorphological studies, the utilisation of techniques of soil sectioning and electron microscopy, and the distribution of microbial communities and their microhabitats. The third chapter gives an account of the distribution of soil animals and microorganisms in relation to the conditions of moisture and temperature in the soil.

It is not until Chapter Four, however, that the main subject of this book is encountered: the structure and species composition of the microbial communities occupying selected habitats of the forest-rendzina soil. A discussion of the current status of the species-concept in the Actinomycetes leads on to a consideration of the techniques of multiple character testing and numerical similarity analysis by which Professor Szabo defines the members of his microflora. In this chapter 34 species-groups from the A horizon of the soil are described using those methods; the description is supported by many pages of tabulation of characteristics and by a discussion of the validity of the status of the species groups in comparison with the conclusions of other workers. In the succeeding two chapters this process is repeated for the actinomycete and bacterial floras of other habitats, notably the gut and faecal communities of the larvae of *Bibio marci*, an important component of the fauna active in soil decomposition, and the rhizoplane community of *Robinia pseudo-acacia*. These three chapters comprise two thirds of the total text; they are almost wholly devoted to the reporting and discussion of the taxonomic studies. This gives a highly specialised character to the book that seems out of keeping with the drift of the early chapters and the stated aim.

The interest of the same readers may, however, be reclaimed by the contents of the last chapter. Here, there is an enormously stimulating discussion ranging over many aspects of the structure and functioning of the microbial communities of soil, including such topics as microbial successions, the role of microbes in soil humification and fertility, the dissemination of soil microbes, and the maintenance of the genetic diversity of soil populations. Here also the ecological implications of the work described in the three main chapters is allowed to struggle free of its taxonomic clothing and to be seen in a wider ecological context.

I was left with a strange impression that I had read two books in one. Anyone with an interest in the microbial ecology of the soil who can gain access to the book will find much to stimulate him in the early chapters and in Chapter Seven. Whether they will find it worth paying the expensive price for the sake of the specialised remainder will depend on their own particular interests. A number of reservations must also be recorded. I found it difficult to be convinced by much of the experimental work in Chapter Three; for instance the relevance of survival testing of microbes over the temperature range 35–65 °C is obscure when related to their activity in a forest soil of temperate climate—particularly when no suggestion is made of potential thermophilic phases. The lack of detail in the reports of the quantitative ecological data contrasts strongly with the admirably complete treatment of the taxonomic data and detracts from the impact of the topics discussed in Chapter Seven.

Nevertheless, this well-produced book remains an important contribution to the literature, partly to the specialised field it serves but more generally because of the opportunity it affords for an appraisal of the Szabo philosophy of "intensive, single-site study".

Mike Swift

Spectroscopy . . .

Laser Spectroscopy. By Richard G. Brewer and Aram Mooradian. Pp. xi+671. (Plenum: New York and London, 1974.) $41.40.

THIS volume contains the proceedings of a Conference held at Vail, Colorado, between June 25 and 29, 1973—one of the most interesting conferences I have had the privilege of attending. The book reports on 45 invited papers and reflects a significant landmark in the application of lasers to fundamental physical investigation in that the impact of tunable laser sources, or related methods, on the spectroscopy of atomic and molecular systems could be seen clearly for the first time. Orders of magnitude advances in resolution and/or spectral brightness were demonstrated and used. The meeting, which was international and interdisciplinary, covered a spectrum from the ultraviolet to the far infrared and microwave regions; it was set in context by addresses covering wider fields of physics by Gerhard Herzberg and Edward Teller.

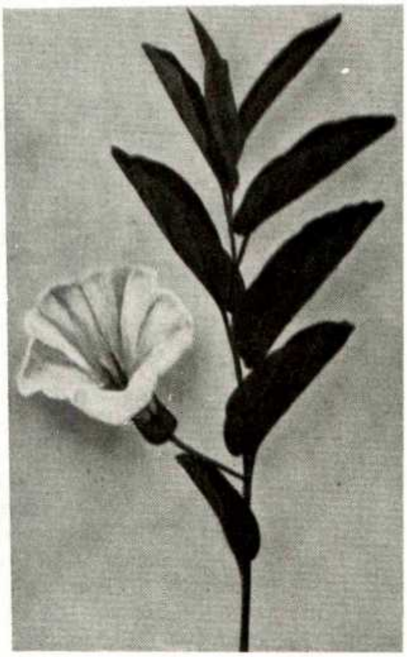
In some ways visible wavelength, dye lasers are the most highly developed: examples of high resolution (50 kHz) atomic beam spectroscopy, spectroscopy of selectively excited atomic states, saturated spectroscopy eliminating Doppler broadening, and quantum beats—modulations arising from the coherent superposition of excited states observed in fluorescence—are variously reported from Cologne, Columbia, London and Stanford universities.

Spectroscopy using tunable infrared lasers is attendant upon the continuing development of the different laser systems. Parametric oscillators ($LiNbO_3$, CdSe) now cover the range from 0.6 μm to 11 μm (but not continuously) and, with mixing techniques, reach 18 μm. As reported by Stanford University, however, resolution is so far disappointing—linewidths are only 0.1–1 cm^{-1}—and good spectra are as yet rare. Four-wave parametric mixing in alkali metal vapours, developed by the International Business Machine company, now covers the range 2–25 μm with rather similar properties.

By contrast, semiconductor diode lasers, until now originating mainly from the Lincoln Laboratory, MIT, collectively extend from 1–32 μm and have yielded many high quality spectra though, individually, they cover only a few wavenumbers. These lasers can now be operated with external cavities. Hyperfine splitting, Λ-doubling and Zeeman splitting effects using laser linewidths sometimes as low as 100 kHz are demonstrated. Progress on Spin-flip Raman lasers is reported from MIT, Bell Laboratories and Heriot-Watt University; this technique retains advantages in its combination of high power and narrow linewidth (from 1 kW at 0.02 cm^{-1}, pulsed, to 1 W at 100 kHz, continuous wave) and is now giving high quality spectra.

Numerous experiments using lasers on molecules and atoms are reported, all reflecting the high precision of measurement now possible. Subjects covered include Lamb-dip spectroscopy, infrared microwave double resonance, precision calibration and stabilisation, heterodyning, laser photochemistry and astrophysical distance measurements. This excellent book is essential for any laboratory concerned with laser spectroscopy.

S. D. Smith



Upright or low bindweed, *Convolvulus spithamaeus.* From *Wild Flowers.* Revised edition. By Homer D. House. Pp. 362+364 colour plates. (Collier–Macmillan; New York, May 1975.) £5.00.

. . . and spectroscopy

^{13}C NMR Spectroscopy: Methods and Applications. By E. Breitmaier and W. Voelter. Pp. xiii+303. (Chemie: Weinheim, 1974.) DM98.00.

THIS book by these authors has obviously grown to adult status from their earlier review in *Angewandte Chemie.* Their child has expanded naturally in each of its parts and has matured considerably but, genes being what they are, is still bound by the same limited outlook, which is strictly organic. Its name, therefore, could be very misleading to the prospective buyer, especially if he detects the strong physical accents of the companion monographs in the family series. Evidently the godfather realised the child's predilections, since his foreword uses the term 'organic' in every other line, but it is clear that the editor ought to have exerted his influence and judgement at the baptism.

The first three chapters give 20 pages of basic, introductory discussion of nuclear magnetic resonance (NMR), 45 pages on methods, and another 45 on the relationships of structure to NMR data, mainly covering shifts (20 pages) and coupling constants (20 pages) with only 6 pages on relaxation times. The introductory first chapter is rather unbalanced—there is much detail, unused later, concerning the Bloch equations, and arguably not enough on complexities in spectra. It contains annoying, but perhaps trivial, mistakes or views; for example, it is stated that shifts, unlike couplings, are not true structural parameters, and there is a misleading labelling of the spin levels in an AX system. A short discussion of relaxation effects in that system would have been useful. Chapter 2 is good but could have been improved by the inclusion of a section on assignment techniques, if only in summary or even tabular form. In chapter 3 the authors acknowledge that their adopted sign convention for shifts is unusual but they give no reason for so sprinkling their handsome volume with a plethora of expensive negative signs.

The second half of the volume is devoted to applications. There are only two chapters, rather arbitrarily divided into "Organic Compounds" and "Natural Products". The volume contains virtually nothing on carbon-containing ligands in either organometallic or other complexes. Discussions of paramagnetic systems and of the phenomenon of chemically induced polarisation are avoided. The latter, even if it had not been extensively applied to ^{13}C studies at the time of writing the book, was clearly going to prove important even then.

The authors have done reasonably well in avoiding the dangers arising from early abstraction of material and slow book production in a developing field but, inevitably perhaps, occasionally important assumptions are not sufficiently emphasised or even explicitly stated—for example the extreme narrowing condition in the Overhauser section, and the symmetry or motional conditions in the lanthamide shift section.

In spite of these criticisms and the high price, the book has attractions. It is very well produced, pleasingly designed, printed and presented, and has 82 excellent figures.

I should like to congratulate the authors on their clarity of expression, especially as they have written in a language which is not their own.

E. W. Randall

from abacus,
through...bubble chamber...
carposporangium...
flying tuck...
pentaerythritol tetranitrate...
and (sic)
uncertainty principle...
to zymetic

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Young men

Essays on the Nervous System. By R. Bellairs and E. G. Gray. Pp. viii+511. (Clarendon : Oxford; Oxford University Press : London, 1974.) £11.50.

THIS admirable book consists of nearly 20 essays by former pupils and colleagues of J. Z. Young—essays well chosen and substantial. They cover most of Young's fields of interest—endocrine activity in the nervous system, by the late Sir Francis Knowles; learning in the octopus, by M. J. Wells; synaptology, by E. G. Gray; the vertebrate retina, by B. B. Boycott—as well as the hard core of the organisation of the central nervous system represented by Zeki on the visual cortex, Webster on vision in the pigeon, Nadel and O'Keefe on the hippocampus, and Ralston on the spinal cord. The peripheral nervous system includes the muscle spindle described by David Barker—a pupil of Young at Oxford before his University College days—growth of nerve fibres in tissue culture by D. W. James; and Hall-Craggs on mammalian skeletal muscle. P. K. Thomas on nerve injury reminds us of Young's war time interests from which grew new lines of immense importance.

It is an enormous range of interests, in which he has touched little without making it flower. Sometimes the flowers take unexpected shapes. In Zeki's work sharp and detailed localisation appears as the ground plan of the various visual re-representations in the prestriate cortex. In the paper on the hippocampus a new and fascinating model is put forward to account for the detailed and regular structure of that puzzling formation.

This is, in fact, the thread that joins up this variety of contributions—the need for detailed and exact knowledge of structure on which to base any account of function in the nervous system. There was a time when random spread and connection of nerve fibres was believed to occur even in the visual cortex. Since then we have learned of the precise limits of eye dominance and orientation columns in the cortex, the sharply defined decussation of fibre tracts in the octopus, and the orderly growth and regrowth of fibres in the optic tracts and tectum. It is arguable that although there have been great advances in knowledge of synaptology from electron microscopic studies of the retina described here by Boycott and more generally by Colonnier, we still need more information at the optical microscopic level. Golgi stains give us a small sample of the cells present but only as individuals isolated from each other. Perhaps what we need more is something which does not yet exist—Golgi-like pictures limited to small groups of cells which are immediately linked to one another, instead of being picked out by the vagaries of staining.

This thread runs through Graziadei's account of the curious regeneration to be found in olfactory nerves, in Guillery's account of experimental production of structural changes in visual pathways, and in Webster's valuable account of the present state of knowledge of visual pathways in birds. Rather oddly, it is not obvious in Wall's essay on cognition and perception as a preliminary to relevant behaviour. What better title could one have for Well's account of the octopus than 'A location for learning'?

This book is a worthy tribute to the work and inspiration of one who has moulded twentieth century studies of the nervous system. **D. Whitteridge**

Applied estimation

Applied Optimal Estimation. Edited by A. Gelb. Pp. 374. (MIT Press : Cambridge, Massachusetts and London; 1974.) $6.95.

THE title of this eclectic compendium is a conventional solecism. 'Applied' means applicable as in 'applied mathematics'. Examples of the use of optimal estimation are included to elucidate the techniques rather than to discuss the application.

A high proportion of computer time in current use is expended on optimally fitting data to models which are ill founded in theory and erroneous in application. Examples abound in every field, from particle physics to town-planning. A mathematically written textbook which sets out conditions under which models can be built up is needed; it may have the same title as this book but this book is not it. The fact that this volume will increase the number of persons technically equipped to estimate model parameters optimally may well turn out to be a misfortune.

But in spite of reservations about what this book is not, it is an excellent handbook of practical, ably described and heuristically presented techniques. It assumes a degree of mathematical sophistication and knowledge which could be reasonably expected in a graduate (the first chapter summarising this information is too breathless to be didactic), treats linear optimisation fairly exhaustively, and makes extensive inroads into non-linear theory. Space is also found for sensitivity analysis and the last chapter on "Additional Topics" includes stochastic estimation and real-time parameter identification.

P. E. Roe

obituary

Arne Westgren, a pioneer in the application of X-ray diffraction to physical metallurgy, and also well-known for his X-ray crystallographic work in other fields of inorganic chemistry, died in Stockholm on March 7, at the age of 85.

Westgren started his academic career in Uppsala as a pupil of The Svedberg and in 1915 obtained the doctoral degree with a thesis on Brownian movement. While employed as a metallurgist at the ballbearing company SKF, he was confronted with several problems which he thought could be solved by X-ray diffraction methods, and found that the α–β transition in iron was not accompanied by any structural change, whereas γ iron showed the same cubic face-centered structure as the austenitic steels at room temperature. These successful results gave Westgren an appointment at the Institute for Metal Research in Stockholm in 1921, where he began an extremely fruitful collaboration with Gösta Phragmén. (Phragmén's great experimental skill resulted in the construction of very efficient X-ray focusing cameras). The studies of the iron modifications were continued and the structure of δ iron established. The unit-cell dimensions of cementite were also determined. In the copper–aluminium system a phase with the γ-brass structure was found for the first time, although its structure was not then solved. The structural analogies in the systems copper–zinc, silver–zinc and gold–zinc were found by Westgren and Phragmén in 1925. At about the same time they investigated the modifications of manganese and studied the carbide systems of chromium, molybdenum and tungsten. They also succeeded in characterising the 'high-speed' steel carbide. The structural analogies between the three systems copper–zinc, copper–aluminium and copper–tin, found by Westgren and Phragmén, gave more solid support to the rule proposed at the same time by Hume-Rothery, and founded on microscopial observations. In 1927 he was appointed Professor of General and Inorganic Chemistry at the University of Stockholm. This resulted in an increasing number of pupils, but nevertheless the collaboration with Phragmén lasted until the latter's death in 1944. The work on metallic systems continued, resulting in more complete structure determinations of phases as well as the solving of a considerable number of new structures. Extensive work on non-metallic systems was also carried out in Westgren's laboratory but was to a great extent performed by pupils of Westgren. In 1943 he was appointed Secretary of the Royal Swedish Academy of Sciences, and from that year only had time to turn to X-ray crystallography in leisure moments. He retired from this last appointment in 1959. Westgren also served as Secretary to the Nobel Committees for Physics and Chemistry from 1926 to 1943 and he was Chairman of the Committee for Chemistry from 1944 to 1965.

announcements

Awards

The prizewinners of the **Feldberg Foundation Awards** are **H. G. Wittman** and **J. B. Gurdon, FRS.**

The Chemical Institute of Canada has announced the following awards: **Fisher Scientific Lecture Award** to **S. Barabas** (application of automated chemical analysis in industry); **Montreal Medal** to **B. B. Migicovsky** ("leadership in the profession of chemistry"); **Merck, Sharp & Dohme Lecture Award** to **L. D. Hall** (synthesising novel carbohydrate derivatives and developing heteronuclear magnetic resonance spectroscopy to study organic compounds in solution); **Noranda Lecture Award** to **B. R. James** (contributions to physical and inorganic chemistry by a scientist under 40); **Chemical Education Award** to **W. E. Harris** (modernising university teaching of analytical chemistry).

The National Acadamy of Sciences (Washington) has announced the following awards: **James Craig Watson Medal** to **G. M. Clemence** (independent derivation of a new analytical theory of the motion of Mars); **John J. Carty Medal** to **J. T. Wilson** (theory of plate tectonics); **Henryk Arctowski Medal** to **J. M. Beckers** (discovery and study of exotic small-scale phenomena in the Sun); **Steel Foundation Award** to **B. M. Alberts** (isolation of proteins required for DNA replication and genetic recombination, and interaction thereof); **Award for Environmental Quality** to **J. T. Middleton** (adverse effects of photochemical air pollution in agriculturally important plants); **Arthur L. Day Prize** to **D. H. Matthews** and **F. J. Vine** (theory of plate tectonics); **Benjamin Apthorp Gould Prize** to **Lodewijk Woltjer** (Crab Nebula and galactic magnetic fields); **H. P. Robertson Memorial Lectureship** to **M. Rees** (high energy astrophysics and theory of quasistellar sources, radio galaxies and X-ray sources).

H. Marion and **G. Mahfouz** have been awarded **Telford Gold Medals** by the Institution of Civil Engineers for their paper 'Design and construction of the Ekofisk artificial island.'

The Royal Society has announced the election of the following as **Foreign Members: H. Gilman** (organometallic compounds and their reactions); **M. Heidelberger** (antigen–antibody interactions and structure of bacterial polysaccharides); **F. Lynen** (intermediary metabolism and enzyme action); **L. Onsager** (statistical physics and chemistry).

L'Académie des sciences, Paris, has elected two foreign associates: **Albert Claude** (Belgium), the winner of the Nobel Prize for Medicine in 1974; and **Joseph Doob** (United States), the mathematician.

Miscellaneous

Research on cancer. The International Union Against Cancer is supporting the Eleanor Roosevelt Fellowships, intended for experienced investigators carrying out independent research at a single institution in another country. Applications and further information (by September 1): International Union Against Cancer, PO Box 400, 1211 Geneva 2, Switzerland.

International meetings

June 23–24, **Scientific and engineering secondary information transfer for developing countries,** Brussels (General Secretary, ICSU AB, 17 rue Mirabeau, 75016 Paris, France).

July 3–10, **International botanical congress,** Leningrad (XII International Botanical Congress, 2 Professor Popov Str, Leningrad 197022, USSR).

July 6–10, **Underwater physiology,** San Diego (Secretariat, 6th Symposium on Underwater Physiology, 9650 Rockville Pike, Bethesda, Maryland 20014).

July 6–11, **Pure and applied chemistry,** Jerusalem (Organising Committee, 25th Congress of the International Union of Pure and Applied Chemistry, POB 16271, Tel Aviv, Israel).

July 6–11, **Nuclear magnetic resonance spectroscopy,** St Andrews, Scotland (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

July 7–11, **Plant pathology,** London (The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Richmond, Surrey, TW9 3AF, UK).

July 8–12, **International committee on laboratory animals,** Thessaloniki, Greece (Dr N. Simionescu, Yale University School of Medicine, Cell Biology Department, 333 Cedar Street, New Haven, Connecticut 06510).

July 8–12, **Phonon scattering in solids,** Nottingham, UK (The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

July 10–11, **Autoradiography,** Edinburgh, UK (Dr J. Jacob, Department of Genetics, West Mains Road, Edinburgh EH9 3JN, UK *or* Dr T. Appleton, Physiological Laboratory, Cambridge CB2 3EG, UK).

July 10–12, **Inflammation,** Nottingham (Dr Alma B. Simmonds, Chelsea College, University of London, Manresa Road, London SW3, UK).

July 13–18, **Enzymatic and homogeneous catalysis,** Santander, Spain (Dr A. O. Ballesteros, Departamento de Catálisis, CSIC, Serrano, 119 Madrid-6, Spain).

July 13–18, **Clinical chemistry,** Toronto (Dr F. H. Sims, Chairman of the Publicity Committee, Women's College Hospital, Toronto, Ontario, Canada).

July 13–18, **Chemotherapy,** London (Conference Services, 43 Charles Street, London W1, UK).

Reports and publications

Great Britain

Safety in Laboratories. Pp. 42. (Manchester: Corporate Public Relations Department, CIBA-GEIGY)UK), Ltd., Simonsway, 1974.) *gratis.* [33

Proceedings of the Royal Irish Academy. Vol. 75, Section A. No. 1: The Diffusion of Small Ions in Air Containing Absorbing Nuclei. By J. M. Golden and P. F. Frain. Pp. 1–10. 30p. No. 2: Small Ion Diffusion Variations in Air Containing Condensation Nuclei. By T. P. Burke and P. F. Frain. Pp. 11–31. 32p. No. 3: Cofinal Biorthogonal Systems and Absolute Schauder Decompositions. By J. J. M. Chadwick. Pp. 33–36. 18p. No. 4: An Algebraic Approach to Classical Spacetime. By P. Yodzis. Pp. 37–47. 28p. (Dublin: Royal Irish Academy, 1975.) [33

The Zoological Record. 1970, Vol. 107, Section 14: Protochordata. Compiled by the Staff of the Zoological Society of London. Pp. 31. 1971, Vol. 108. Section 1: Comprehensive Zoology. Compiled by the Staff of the Zoological Society of London. Pp. 64. £3.75. Section 5: Echinodermata. Compiled by the Staff of the Zoological Society of London. Pp. 80. £6. Section 10: Crustacea. Compiled by the Staff of the Zoological Society of London. Pp. 125 £10. Section 16: Amphibia. Compiled by the Staff of the Zoological Society of London. Pp. 106. £7.50. Section 17: Reptilia. Compiled by the Staff of the Zoological Society of London. Pp. 124. £7.50. (London: The Zoological Society of London, 1974.) [33

Heriot-Watt University, Edinburgh. Annual Report, 1973/1974. Pp. 84. (Edinburgh: Heriot-Watt University, 1975.) [43

Concorde: The Questions Answered. Pp. 48. (Weybridge, Surrey: British Aircraft Corporation, 1975.) *gratis.* [43

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The Royal Greenwich Observatory: An Historical Review Issued on the Occasion of Its Tercentenary. By William Hunter McCrea, FRS. Pp. 80 + 12 plates. (London: HMSO, 1975.) £1 net. [73

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Other Countries

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nature

May 15, 1975

Are the art treasures and the overseas students safe?

LAST week it was Mr Reg Prentice's turn, as Secretary of State for Education and Science, to appear at the House of Commons Select Committee's hearing on the financing of scientific research in British Universities. To his sympathetic questioners he declared that the universities were marginally more able, twelve months ago, to bear cuts in their funding than were most other parts of the educational system. There had been a generosity in the staff-student ratio which could bear a little squeezing, but now that had been done, and he did not think one could any longer generalise on the ability of universities to stand further onslaughts. But perhaps some well-endowed establishments might sell off a few of their oil-paintings to tide them over the crisis. The British Antique Dealers' Association pronounced the idea "morally reprehensible".

If we are down to dusting off the art treasures, then we are also at the level at which some other appurtenances of university life are vulnerable. The committee has already had a nose around unproductive research without much in the way of enthusiastic response from its witnesses. Where it does seem to be generating some momentum of its own is on the issue of postgraduate students from overseas. Thus Mr Norman Tebbit asked Mr Prentice whether overseas students were not occupying too much of the "well found" floor space supported by the University Grants Committee (UGC) and hence the taxpayer. Mr Prentice gave the standard deadbat response that there were long-term diplomatic and commercial benefits to having overseas students here.

It was clear that there is a view on the committee not only that overseas students could be charged rather more for their time here but also that there are some cases where the benefits of having overseas students are mainly benefits to the sending countries. This view is helped along by two observations: first that there are departments, particularly in the applied sciences, where overseas postgraduates are in the majority and, second, that although nobody is questioning the concept of aid to developing countries through the provision of university education, a growing number of postgraduate places is being taken up by students from the developed world.

The concern is a reasonable one within many people's concept of what a university should be, and deserves serious attention. In the five years from 1968 to 1973 the number of those obtaining higher degrees (in all subjects) has grown steadily from around 10,000 to around 14,000 a year. (Roughly 40% of these degrees are doctorates.) While the number of Commonwealth higher-degree recipients has remained static at about 1,500, the numbers from other countries has doubled from 1,000 to 2,000. In 1973, 27% of all recipients of higher degrees were from overseas—in pure science 22%, in applied science 36%. About 60% of overseas students returned home after obtaining their degree. In the same year 376 first-degree British graduates went overseas for further education; this figure has declined from 538 in 1970. Most recent figures show that overseas students now account for 33% of postgraduates and applied sciences probably over 40%.

Britain gives more than she takes in higher education, and fairly clearly she has been giving more to students from developed countries in recent years. Is there a case for any control of numbers? The reasons normally advanced against the suggestion are not the most compelling.

Although it is true that the training of students here generates a lot of goodwill in academic, industrial and governmental life around the world, it would be difficult to demonstrate that this creates much in the way of hard returns beyond providing professors with a choice of venue for their sabbaticals. If postgraduate training increases sophistication in students, it must be assumed that they are unlikely to be swayed in the purchase of, say, some piece of equipment by the fact that they went to university in the country in which the makers are situated. And if a piece of equipment being considered is one with which they had experience when in Britain, it is entirely possible that it was imported into Britain or did not prove satisfactory (or both).

The diplomatic profit argument is no easier to sustain. Armies lead by Sandhurst-trained officers have been known to act in other than Britain's interests. Is there any evidence that London-trained ministers of trade, technology, communications, oil and so on look any more kindly on the country in which they obtained their postgraduate education? Any Harvard-trained minister in the British government who started favouring US business would soon be out on his ear.

There are two much better arguments for encouraging the flow of overseas students—one idealistic, one chauvinistic. On the idealistic plane, Britain supports universities because they are places of learning, and learning, even in the applied sciences, knows few frontiers. True, the man with the key patent will profit himself and his own country first, but the benefits very quickly flow to the users as well as the inventor. And the inventor will have had to dip into a truly international pool of knowledge. Thus talk of great new British inventions, so beloved of newspapers, is often meaningless. The more the international character of learning is stimulated by the interchange of people, the more broadly and effectively does the academic life operate. (But fewer Britons are going abroad for postgraduate education.)

On the chauvinistic level, every postgraduate who comes to Britain is potentially capable of making a significant intellectual contribution even within a three-year period—and many do. It is often forgotten that some do their best work when still a student, and this work is then immediately available in the British community. Besides which, many stay for the rest of their lives. □

ABBAS

Famine revisited

Recent reports from Ethiopia indicate that famine has again struck the pastoral population of the south. A year ago an article in Nature *made the case that the collection of objective information in droughts is of the first importance in planning relief operations. The London Technical Group has undertaken the design and conduct of such surveys in Ethiopia and elsewhere. In this article Julius Holt, John Seaman and John Rivers of that organisation discuss their observations made on the pastoralists and farmers of Harerge Province, South-east Ethiopia, in mid-1974 after the first wave of drought. They comment on the failure of relief efforts to prevent death and disruption when the rains again failed.*

POPULATIONS do not suddenly starve; but starving populations becoming sudden and dramatic news. The process leading to famine after a natural catastrophe is prolonged and complex and occurs against a background of endemic malnutrition and poverty. By the time a famine is internationally recognised it is already at or past its peak, and relief aid does not usually begin to arrive in significant quantities until the starvation crisis is over and the majority of famine deaths have occurred. Emergency aid is, moreover, disproportionately costly, and economic considerations therefore support the humanitarian reasons for investing in timely and informative surveys to warn of developing famines and to guide effectively in their relief.

A year ago, workers from the London Technical Group and the Medical Research Council Dunn Nutrition Unit described in *Nature* their experiences in the drought-affected areas of north-eastern Ethiopia, pointing out the need to develop standardised survey techniques both for rapid famine assessment and for monitoring the situation during the danger period. By that time the focus of the drought was no longer in the agricultural highlands of the north-east but had shifted to predominantly pastoral regions, particularly the vast rangelands of Harerge Province in South-east Ethiopia. Some reports of starvation emanated from that area as early as the end of 1973.

The importance of rapidly gained but systematic information had won increasing recognition among relief agencies. Members of the London Technical Group were invited by the Ethiopian Relief and Rehabilitation Commission to design and direct a survey of Harerge from May to July 1974, and the project was supported by UNICEF, USAID, Oxfam and Christian Aid.

The purpose of the survey was twofold: the most urgent objective was to discover as rapidly as possible the nutritional condition of the population, but it was also hoped that the information gained would form the basis of a longer-term surveillance scheme. The problems presented were not inconsiderable. The geography of the region to be surveyed was daunting, for Harerge, which shares an extensive border with northern Somalia, is larger in area than England but has an extremely poor road system. A highland ridge, supporting an agricultural population of some 2.2 million, cuts into a semi-desert lowland ('Issa and Ogaden) which is seven times greater in area, with a population in normal times of up to 0.5 million pastoralists. There was a profound lack of baseline data on the area, which made it impossible to relate the survey sample accurately to the population distribution and thus made it necessary to include a large retrospective element in assessment.

The logistical difficulties were greatly reduced by the provision of three helicopters by USAID which, with some ground transport, allowed survey teams to make overnight stays at 65 sample sites within a month. But the difficulties inherent in the lack of baseline data remained largely intractable, especially since the effects of the drought were at the time of survey less dramatically obvious than some had expected.

The survey of the nutritional status of more than 1,000 children showed that both pastoral and agricultural populations were generally in a poor nutritional state, but severe protein-energy malnutrition was exceedingly rare. During the preceding twelve months death rates reported to us for children, particularly those of pastoral groups, had, however, been far higher than any reasonable estimate of mortality in a developing country would lead one to regard as normal, and the current demographic picture supported these reports. It was clearly not possible to be certain of the causes of death, but we were satisfied that no epidemic had occurred, and there seemed no reason to doubt the statements of the people that the chief cause had been malnutrition.

Experience elsewhere had led us to suspect that we might indeed be faced with a population which had suffered a tragedy but which at the time of the survey would not exhibit signs of extreme undernutrition. Much of the enquiry was therefore directed to a rapid attempt to define the impact of the drought on food availability for the near future. The Ethiopian Ministry of Agriculture had reported patches of poor harvest in the highland areas of the province, with some serious losses among the cultivators of the lower hills. Local people reported extremely high losses of livestock (cattle, sheep, goats and camels) and systematic observations during the survey of livestock held by pastoral groups showed that herds and flocks held were at about the number which had been reported as the minimum necessary for survival in the area. Before the main 'Gu' (March–May) rains, which had occurred just before the survey, these had been in poor condition and their lowered milk production could well have caused much of the mortality.

But the problem had been compounded by the fact that grain prices had risen steeply, and the survey found that rises were particularly extreme in the Ogaden where grain had been unavailable in some markets at times during the year. The significance of this is that the pastoralists live not only on the produce of their animals but also by selling them in order to buy grain. At the time of the survey the combination of livestock losses, the fall in the cash value of animals and the rise in the cost of grain had reduced the effective wealth of many groups to less than 20% of the level of the previous year and to under 10% of that of times before the drought. During the height of the drought some animals had been unsaleable and herders had been forced to eat animals they were no longer able to keep alive. The nutritional implications of this are considerable: for example, a heifer sold for grain would normally buy at least 8 times as much food (in calories) as are available from its meat.

The survey showed that the physiological reserves of this population were low and that their economic reserves had fallen to a point which in many instances was not far from that of no return. In short, this was a highly precarious nutritional situation. But the implications for relief were problematic. Grain is the basic currency of relief for pastoral as well as agricultural populations. But the logistics and administrative difficulties of distributing grain to a mobile population have never been successfully solved. The scheme actually adopted relies on the encampment of groups round administrative centres, thus simplifying the transport of grain. The result is large congregations of women and children together with some aged people; the men continue to move with the animals. There are three inherent dangers in this: first, the threat of long-term disruption in the social lives of families and groups; second, the risk of epidemic disease in such large congregations; third, the possibility that groups will not arrive in centres in time to avoid some mortality. The temptation under stress for whole groups, including the men, to become entirely dependent in the long-term on grain hand-outs is of concern to the authorities, and has important implications for the future of the pastoral economy in an area where agriculture will not be a viable alternative at least for many years to come.

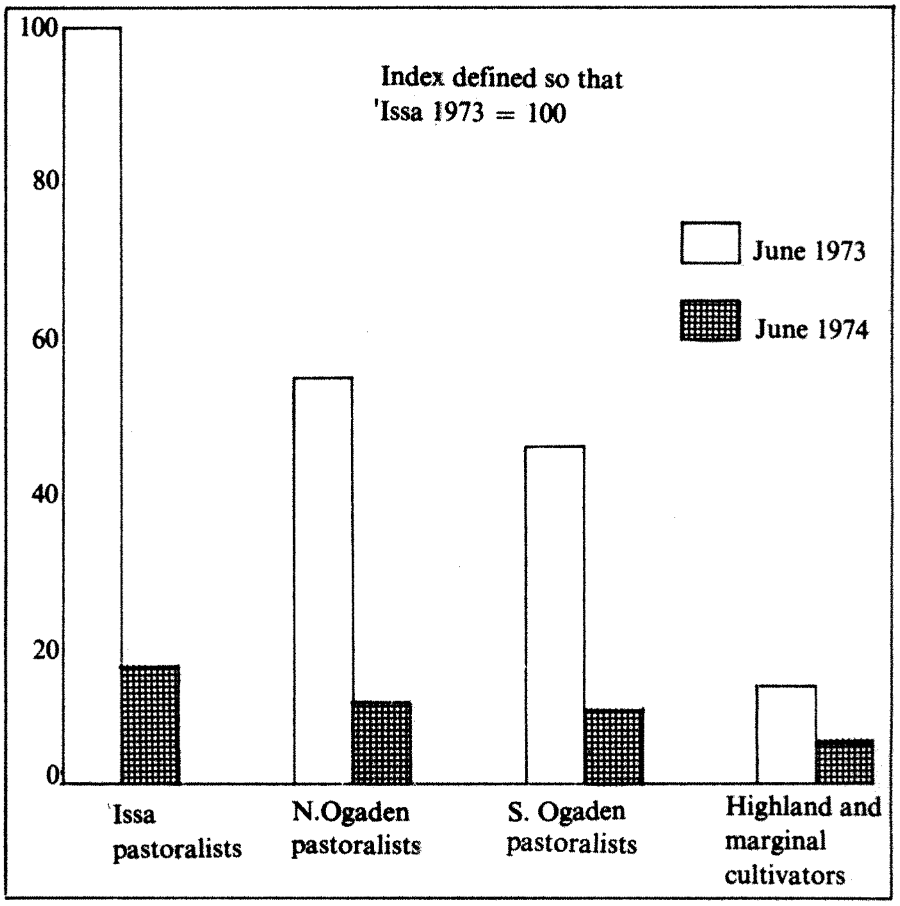
At the time of the survey we concluded that a further failure of the short rains in September–October 1974 was likely to be catastrophic for the pastoralists, whereas normal rains would allow them to take a vital step in strengthening their self-sufficiency. The recommendations, therefore, were that although resources should be husbanded for a major relief operation in the event of rain failure—by stock-piling grain and making provision for the large amount of transport which would be required—the other urgent priority at that time (July 1974) was the institution of a surveillance programme to monitor changes in the situation. Unfortunately the kind of sustained field-surveillance originally envisaged did not materialise. Meanwhile, a limited relief programme continued for the relatively small numbers of destitute people who were then clustered around centres.

In the event the autumn rains were sparse. Reports reaching us from the Ogaden in the first part of 1975 spoke of a deteriorating situation with numbers around relief centres (mostly women and children) growing to 50,000. A recent report from Oxfam cited a peak death rate in this group of 150 children a day, which, in spite of the efforts of medical teams, had not fallen below 50 a day by early April. Such numbers of people in such condition offer a disturbing indication that the linked problems of surveillance and effective relief there are far from overcome.

Most estimates of food availability in developing countries depend on ratios of estimates of crop yield and spoilage to estimates of population, and are subject to large errors. In particular, the sensitivity of such ratios as indicators of change is poor. Prediction of famine is only possible at the most general level by such techniques, and it is impossible to pick up the smaller local shifts between hardship and disaster. On the other hand, a comprehensive assessment of a famine situation has been shown to be possible using the kind of nutritional and socioeconomic measurements that formed the basis of the survey described here, with the special advantage that, provided care is taken with the sampling technique, the accuracy of the system is sufficient to indicate relatively small changes in the nutritional status of local populations.

Baseline surveys and the organisation of repeat surveillance should be considered a priority in those areas of the developing world which are now identifiably prone to extreme localised food shortages. This would not constitute a large diversion of resources. For instance, in spite of its size the Harerge survey cost, helicopters apart, less than £10,000. In subsequent surveillance of such areas the body of the work does not need highly trained personnel or a high capital investment in sophisticated equipment. The use of expensive air transport tends to be the price paid for belated reactions to an emergency. In any of the recent African droughts an effective field-surveillance programme could have been mounted for less than 1% of the cost of the total relief programme, and could have helped to ensure that for the first time aid arrived before people died of starvation. ☐

Purchasing power of animal herds in grain equivalents.



What kind of strategy for leprosy?

By S. G. Browne, Director of the Leprosy Study Centre, and T. F. Davey, of Leprosy Review.

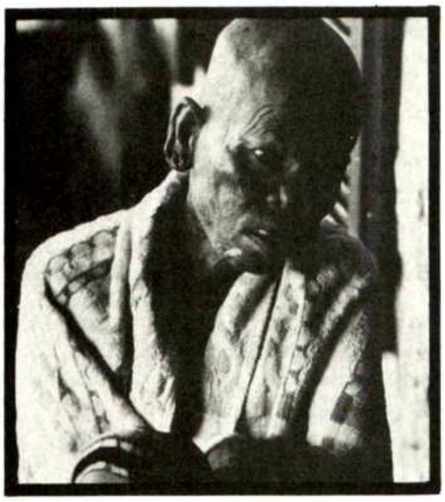
AN article by C. L. Crawford (*Nature*, March 20), stressed in vigorous terms the well recognised concept that the best available means of secondary prevention of leprosy lies in the regular and sustained treatment of leprosy sufferers with the cheap and effective mycobacteriostatic drug, dapsone. We agree, and have said the same, many times.

The well-night insuperable difficulties facing the World Health Organisation and voluntary agencies, in trying to implement a strategy on which they are now generally agreed, are concerned with factors to which Crawford seems to pay little attention in his article. These factors are, on the one hand, the social aspects of leprosy, their effect on government policy and priorities, and patient attitudes to leprosy, and, on the other, the inadequacy of diagnostic and curative facilities in precisely those countries where leprosy constitutes a major health problem. Add to this the long duration of treatment advised and the need for regularity of treatment—if deformity is to be prevented and the risk of drug resistance minimised—and the world situation can be seen in its stark reality, which is reflected in successive reports from the World Health Organisation and its Expert Committee.

Those of us who have been concerned for upwards of 40 years with mass treatment schemes for leprosy and ambulatory treatment/control measures integrated into the general urban–rural health services cannot but agree that "sulphones provide a cheap and practical form of treatment and also prevent transmission of leprosy". A recent editorial in *Leprosy Review* says the same thing: "The maximal extension of sulphone treatment, and a skilled sustained campaign of leprosy education are priorities for us all." The problem is to discover large scale schemes, adequately documented and supervised, in which this thesis can be conclusively demonstrated. An oversimplification of his case, illustrated by atypical populations with "a small leprosy problem" and highly selective official estimates of incidence rates, may not convince the cautious epidemiologist.

It would be a salutary exercise to expose the deficiencies in Crawford's article: "the mode of transmission and the incubation period are unknown"; "there is no . . vector to spread the disease"; "only one in ten of the exposed population, at the most, acquire the disease"; leprosy declined in Norway "by a policy of segregation"; "once eradicated from any country, it has never returned"; "no attempt has been made to obtain information about incidence rates"; "accurate figures of leprosy are available . . . in Rumania, Cyprus and Libya".

From recent personal observations, we consider that the conclusions drawn by Crawford regarding the situation in some of the countries he refers to are not in accordance with the facts; on the contrary, the total prevalence rates (comprising the accumulated back-log of high incidence rates associated with a virtual breakdown of rural health services) are much higher than they were 15 or 20 years ago. This does not, of course, invalidate his main thesis that regular and adequate sulphone therapy could eventually result in a reduction of incidence rates, but it does emphasise the realities of today's world and the intractability of the problems of the control of transmissible diseases, among which leprosy figures along with malaria, tuberculosis, trypanosomiasis, onchocerciasis, schistosomiasis and the rest.



Courtesy The Leprosy Mission

Crawford asserts that "drug resistance is very rare". It was, but it is not now, and the recent figures coming from countries where the problem has been adequately investigated are very disturbing, to say the least.

"In many (countries) the leprosarium remains the only place where treatment can be obtained". This statement is hopelessly out-of-date, as is the incredible assertion that, of the £2 million raised annually by voluntary agencies in Europe for leprosy work, most is still spent on institutional care. More than 900,000 leprosy sufferers are being treated at the moment through programmes assisted financially by these maligned voluntary agencies, and the vast majority of these patients are being treated in ambulatory schemes; in addition, about £250,000 a year is being devoted to leprosy research. About a third of all leprosy patients now receiving any treatment at all are getting it through these same voluntary organisations. If such mis-statements are allowed to pass uncorrected, a grave disservice will be done to the cause of truth and to the leprosy sufferer.

As for prophylaxis, to judge by the tempo of much cooperative research based on the availability of relatively enormous quantities of *M. leprae* obtained from experimentally infected armadillos, a specific vaccine should be produced in the not-too-distant future, together with a specific skin test. Chemoprophylaxis (with dapsone) has been shown to be effective, as Crawford points out, but here again the practical objections to large scale implementation are over-riding. Countries where such a measure might be justifiable on general grounds lack both the medical infrastructure and the finance, not to mention the cooperation of the population necessary over a prolonged period (possibly 3–5 years) during which the regular ingestion of a toxic drug by mouth (dapsone) or by intramuscular injection (acedapsone every 75 days) would be necessary.

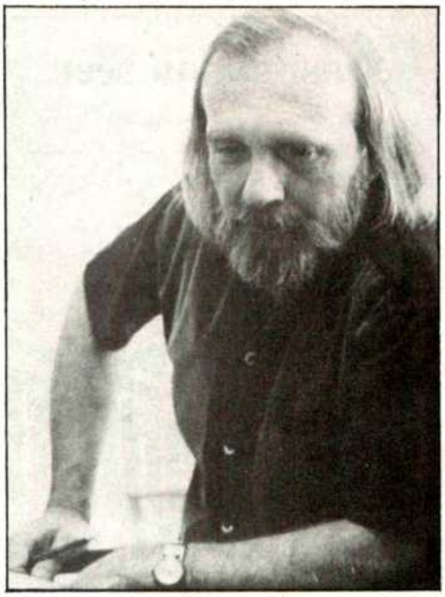
Ministries of Health the world over are facing the problem of a chronic crippling disease, which, ideally and considered in isolation, might require a special service with vast resources—for diagnosis, laboratory cover, prolonged treatment (with a drug that is cheap, admittedly, but whose real cost is greatly enhanced by the expense of getting it to the patients who need it), preventive physiotherapy, provision of protective footwear, not to mention rehabilitation facilities. The social and medical dangers of over-emphasising one of the endemic diseases lie in creating or perpetuating the idea of 'uniqueness' and 'difference' and hence encouraging the stigma attached to the disease.

If treatment of leprosy could be rapid as well as cheap, unaccompanied by severe episodes of reaction as well as effective, capable of bacillary clearance as of mycobacteriostasis, then Crawford's fundamental thesis would be gladly accepted by governments. Meanwhile, inadequately staffed and inadequately financed health services in many countries in the Third World, while awaiting a more effective strategy of control based on primary prevention of leprosy, are striving with varying degrees of enthusiasm and success to put into practice the principles of secondary prevention based on the availability of a cheap and reasonably effective drug, dapsone. ☐

international news

Scientific freedom and responsibility

by Colin Norman, Washington



Tamplin: troublesome scientist

DURING the late 1960s, two scientists employed by the Atomic Energy Commission (AEC), John Gofman and Arthur Tamplin, launched a public attack on the AEC's regulations governing permissible standards of exposure to ionising radiation. They contended that the regulations were too lax because people exposed to radiation at the permitted levels would run an unacceptable risk of developing cancer. The AEC responded in kind with a vigorous public defence of its regulations. But, according to Gofman and Tamplin, it also responded with a private attack on its two troublesome scientists — it greatly reduced their laboratory budgets and staffs.

That episode highlights a problem which has been cropping up with increasing frequency, namely, how can scientists who feel a moral obligation to speak out on particular issues do so when such actions place them in direct conflict with their employers or with other powerful vested interests? And that particular episode also provided a catalyst for the establishment by the American Association for the Advancement of Science of a committee to investigate such conflicts.

The commitee's report finally appeared last week after an inordinately long gestation period of 4½ years. Written for the committee by John T. Edsall, a Harvard biologist, it stands out as a thoughtful and eloquent statement on academic freedom and scientific responsibility—a field which has all too frequently been a subject of much academic claptrap.

First, Edsall discusses some of the moral and ethical concerns which have arisen among scientists themselves over a few areas of research, and which could result in restrictions on academic freedom. Noting that "the basic function of the scientific community is the advancement of knowledge", he suggests that some scientists "might well believe that nothing should be off limits as a possible subject for basic research, as distinct from applied science and technology". But he reckons that "there may be strong reasons for declaring certain areas of research off limits, or at least for imposing severe restrictions on them".

Some types of experiment on human subjects, for example, "raise serious questions" and should be carried out only according to strong ethical and practical guidelines which may inevitably rule out some of the more dangerous activities. Moreover, the recent call for a temporary halt in some types of genetic manipulation experiments was "a landmark in the assumption of scientific responsibility by scientists themselves for the possible dangerous consequences of their work".

But, on more controversial ground, Edsall offers a strong defence of the legitimacy of research into the role of genetic factors in human intelligence. It has been argued that such research should be declared out of bounds because it could exacerbate racial prejudices. But Edsall strongly supports "the view that inquiry into genetic differences between racial groups is a thoroughly legitimate field of research, and we condemn the emotional attacks that have been made against such persons as Arthur Jensen and R. J. Herrnstein, because of their views concerning the importance of genetic factors in human intelligence. Their views are, of course, open to critical debate and challenge on scientific grounds, as long as the debate is conducted on an objective scientific level".

The problem, however, is that once such views enter the political arena, the debate automatically leaves the "objective scientific level", and the real question is what responsibility scientists should have for the uses to which their research findings are put. On the particular question of racial differences in intelligence, Edsall offers only the suggestion that "it is wise for investigators to state their results cautiously and modestly, to specify clearly the limits of accuracy, and to refrain from sweeping claims based on highly specialised researches".

As for conflicts which arise when scientists blow the whistle on their employers or make public statements on scientific issues which place them in conflict with vested interests, there are many examples of the subjection of scientists to professional harassment for trying to defend what they see as the public interest. The case of Gofman and Tamplin is one example. Another is the case of three engineers working on San Francisco's Bay Area Rapid Transit System. In 1972, they were summarily dismissed, allegedly for making statements about defects in the train control system—statements which later turned out to be correct.

There have also been occasions when scientists working for industrial concerns have sided with the company in suppressing data damaging to their employers' operations or in resisting changes which would result in more rigorous health and safety standards.

In such instances, Edsall argues that professional societies should play a much more active role in defending the interests of their members or in adjudicating in disputes where issues of scientific freedom are involved. The societies "can deal with such issues by setting up committee of inquiry, in cases where a serious violation of scientific responsibility is suspected; by publicising the results of the inquiry in professional journals, and, if necessary, in the more popular journals and in the news media; and by calling the matter to the attention of governmental bodies", Edsall suggests, and he adds that "they can on occasion launch law suits on behalf of members who have apparently suffered injustice when acting on behalf of the public interest".

Although such activities may raise serious problems of funding and thus would require individuals to spend substantial amounts of time serving on hearing panels, Edsall reckons that "increased activity of the professional societies [is] the most hopeful approach to the problem in the immediate future". □

Washington seen

from Colin Norman

A SET of figures, published last week by the Department of the Interior, casts further doubts on the ability of the United States to shake off its dependence on imported oil. The department has drastically reduced its estimate of the amount of recoverable oil and gas that remains to be discovered in the United States and the new figures suggest that domestic resources could be severely depleted within about 30 years.

The new estimates are close to those published earlier this year by a committee of the National Academy of Sciences, on the basis of which the committee concluded that "US independence from external sources [of oil and gas] is essentially impossible on the basis of increased production of petroleum during the next decade".

The Department of the Interior now estimates that there are between 50×10^{9} and 127×10^{9} barrels of oil and between 320×10^{12} and 655×10^{12} cubic feet of natural gas yet to be discovered within the United States and, in deposits offshore. In addition, it reckons that about 30×10^{9} barrels are recoverable from unexplored parts of existing fields.

Those figures stand in sharp contrast to estimates published a year ago which suggested that undiscovered oil reserves amount to between 200×10^{9} and 400×10^{9} barels; gas reserves were put at between 990×10^{12} and $2{,}000\times10^{12}$ cubic feet. Moreover, in 1972, the Department of the Interior was predicting that undiscovered recoverable oil reserves amounted to 458×10^{9} barrels. Those earlier estimates have, however, come under sharp attack from the oil industry because they were based on allegedly unrealistic estimating techniques, and the new estimates are derived by a method which the oil companies—and the committee of the National Academy of Sciences—believe is more accurate.

If the new estimates turn out to be correct, they hold a number of very important implications for energy policy in the United States. First, they dramatically underline the need for increased conservation of oil and gas. And, second, they make more urgent the need to develop replacements for natural gas and petroleum, such as synthetic fuels produced from coal. As Dr Vincent E. McKelvey, Director of the US Geological Survey, which developed the estimates for the Department of the Interior, put it last week: "[they] show that it is necessary soon to develop other sources of energy as the mainstay of our future energy supply".

● In a move hailed as a major victory by environmentalist organisations and as a major tragedy by nuclear power industry, the Nuclear Regulatory Commission (NRC)—which recently assumed the regulatory functions of the defunct Atomic Energy Commission—has announced that it will postpone, for up to three years, a final decision on whether plutonium will be allowed as a fuel for nuclear power plants in the United States.

Plutonium, which is a by-product of the fission reaction in nuclear power plants, could be recycled as a nuclear fuel to help eke out domestic supplies of uranium in the United States. The former Atomic Energy Commission had hoped to make a final ruling this year on whether or not plutonium recycling should be allowed, but the NRC has now decided that the whole matter should be the subject of public hearings.

According to critics and supporters of nuclear power alike, the NRC's final decision on plutonium recycling will be crucial to the development of the nuclear power programme. For one thing, plutonium recycling would more than double the amount of energy which could be extracted from a given amount of uranium and it would therefore help hold down the costs of electricity production. And, for another, part of the justification for the breeder reactor rests on the fact that he plutonium produced in breeders would be used to fuel light water reactors. As Thomas Cochran, who works for the Natural Resources Defense Council, pointed out last week, "the breeder programme would look a bit silly if plutonium were banned as a fuel in light water reactors".

The chief objections to plutonium recycling are that it would involve transporting large amounts of plutonium around the United States. Since plutonium is highly toxic, and since it could be used as the core of a nuclear explosive, opponents have argued that plutonium recycling would increase health hazards and greatly elevate the risks that a terrorist group could make off with a few critical masses of potential bomb material.

Environmentalists are pleased with the NRC's decision to hold public hearings before making a final ruling, because they are convinced that the delay will allow opposition to increase. The nuclear power industry, on the other hand, is less enthusiastic. Mr Carl Walske, President of the Atomic Industrial Forum, last week called the decision "ironic and deplorable", particularly in view of the Interior Department's recent drastic reduction in its estimates of oil and gas reserves in the United States.

The decision to postpone a final ruling on plutonium recycling may, however, turn out to be a shrewd political move on the NRC's part. It was the first major decision taken by the commission, and by opening up the issue to further public debate the NRC has served notice that it is prepared to take a tough stand in spite of vocal opposition from the nuclear industry. Its predecessor, the Atomic Energy Commission, was constantly accused of promoting the nuclear industry instead of regulating it.

● The National Aeronautics and Space Administration is moving into the final stages of planning a space mission to Uranus in the 1980s. Last week, NASA invited scientists to propose experiments for the mission, which would probably be launched in 1979, swing past Jupiter in 1981 and reach Uranus in 1985.

As now planned, the mission would use a Mariner-type spacecraft, which would pass Jupiter at a distance of about 392,000 kilometres and come as close as 24,000 km to Uranus.

Because Uranus has many interesting and enigmatic features—such as the fact that it spins on its side and its atmosphere seems to contain large amounts of methane—the mission has strong support among space scientists. But, earlier this year, the National Academy of Sciences suggested that NASA should reconsider the mission's priority in light of the fact that the space science budget is unlikely to grow much, if at all, in the coming year. Officials of NASA believe, however, that the mission could be fitted into the budget for two reasons. First, it will rely partly on existing hardware, which will keep the costs down and, second, the major expenses will be incurred before the most expensive development phase of other space missions such as the Large Space Telescope and a possible Jupiter orbiter mission.

Porton lab will study fever virus

by Eleanor Lawrence

THE new laboratory at the Microbiological Research Establishment, Porton Down, formally opened last week by Lord Godber, provides facilities unrivalled in Western Europe for the safe handling of the most dangerous viruses known.

The new facilities will be used first to study the Lassa fever virus, and the other haemorrhagic fever viruses which have mainly been discovered within the past ten years and are virtually untreatable.

Porton, with a long history of handling some of the most dangerous organisms known to man was the obvious location for such a centre. The new laboratory was started two years ago, and has been modelled on facilities at the Communicable Diseases Center at Atlanta, Georgia, which, until now, was the only laboratory considered sufficiently secure to handle the Lassa fever virus.

The first task of the laboratory will be to build up a bank of diagnostic sera against the rare haemorrhagic fevers, such as Bolivian and Congo haemorrhagic fevers, and other exotic tropical virus diseases, such as Marburg disease, that may be brought into the UK. It will also provide this facility for other European countries and, in conjunction with Atlanta, for the areas where these diseases are endemic.

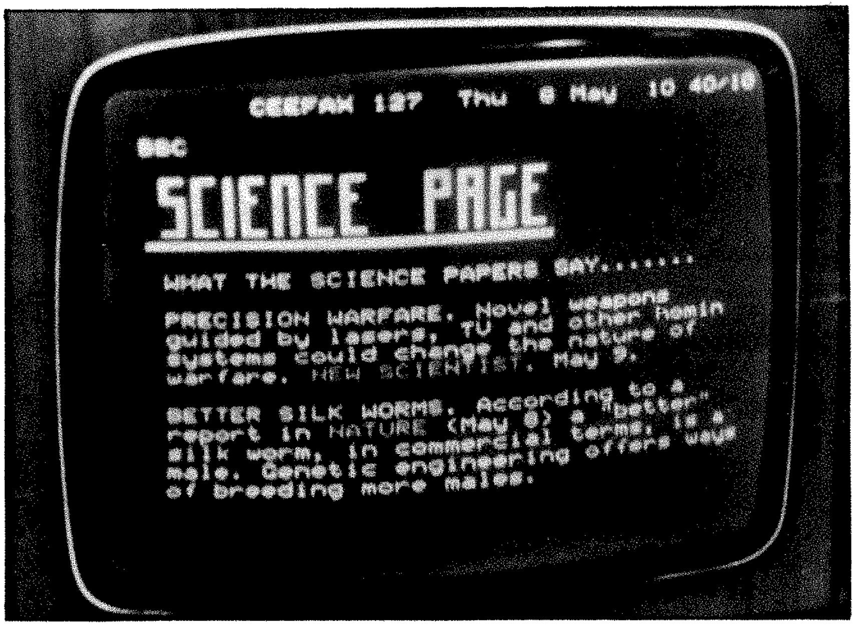
Lassa fever (first recognised in Nigeria) seems to be one of the most virulent virus diseases yet discovered. The mortality in Europeans with diagnosed Lassa fever is around 48% (although it seems to be lower in the native Nigerian population). It is also one of the most infectious virus diseases known. Patients shed virus from all body secretions and through the respiratory tract, and specimens of blood and urine are heavily infected. When the virus is multiplied up in tissue culture or in experimental animals, the risk of infection is greatly magnified.

Apart from its primary use in dealing with the virulent tropical viruses which may be carried into the UK undetected at any time by people returning from abroad, the laboratory will undoubtedly be in demand from research workers wishing to carry out some of the more potentially dangerous genetic engineering experiments in which the DNA of bacteria or viruses is modified by the addition of foreign DNA from other bacteria, viruses or even higher animals. Professor Robert Harris, the Director of Porton, said last week that he had been approached by the Ashby Committee and had argeed that the laboratory facilities could be placed at the disposal of research workers wishing to undertake such experiments, but that he had not yet received any specific proposals. As the laboratory is sufficiently secure to satisfy the most stringent criteria set down by the recent conference called by Professor Paul Berg in California early this year, proposals will probably not be long in materialising.

The Berg conference recommended that experiments in which hybrids of two different viruses were made, or virus DNA (or DNA transcribed from RNA viruses) was linked to bacterial plasmids to multiply up virus genes, or mammalian DNA was linked to bacterial DNA could be carried out under conditions slightly less stringent than those in the new laboratory at Porton but that where the hybrid or the starting organisms were virulent or likely to prove so, the maximum security precautions should be taken. The other proviso of the Berg recommendations was that genetically enfeebled strains should be used.

But the prime object of the new facilities is the identification of the rare but virulent tropical viruses, which pose not just a potential but an actual public health hazard. With aeroplanes bringing their human and animal cargo into European airports every day from the tropics, the possibility of an epidemic of one of these virulent untreatable viruses cannot be discounted.

The only specific therapy which has proved effective against Lassa fever is serum taken from a convalescent patient. But with only three Europeans having recovered from the disease, the supply of convalescent serum is extremely limited. Also, administering convalescent serum from a newly recovered patient can be extremely risky as virus is excreted from the patient for several weeks after clinical recovery. One aim at Porton is to establish a bank of safe serum, either from convalescent patients or from the naturally resistant native population, which can be given to patients once the disease has been diagnosed. □



CEEFAX 127 Thu 8 May 10 40/18

BBC

SCIENCE PAGE

WHAT THE SCIENCE PAPERS SAY......

PRECISION WARFARE. Novel weapons guided by lasers, TV and other homin systems could change the nature of warfare. NEW SCIENTIST, May 9.

BETTER SILK WORMS. According to a report in NATURE (May 8) a "better" silk worm, in commercial terms, is a male. Genetic engineering offers ways of breeding more males.

THE BBC has been providing at first 24 and now 50 televised "pages" of printed material by using spare lines in the ordinary BBC 1 TV channel since last September; the service (code-named CEEFAX) is soon to be increased to 100 pages, and a similar service (called Oracle, but using the same standard technology as CEEFAX) will be provided by the Independent Broadcasting Authority (IBA) from next month. Until recently, the cost of a decoder to enable these printed words to be read on an ordinary television set has been prohibitive—at least £300 for a custom-made box of electronic tricks. But last week Texas Instruments, Bedford, announced in London that they will be producing appropriate decoders on a production-line basis by early next year, when they should be available for about £50. A television set with such a decoder built in at the construction stage should then cost about £100 more than an equivalent set without a decoder, and the industry hopes that prices will fall.

As well as newsflashes, sports results, a science page (see above), weather maps and other services already available from CEEFAX, this new technology opens up intriguing possibilities. Using telephone links and a keyboard, this kind of 'teletext' could help to create a home computer terminal, and there are obvious potential applications in education.

Trying to get MPs interested in science

from David Spurgeon, Ottawa

FIVE years ago, when interest in science policy was still running high among some Canadian scientists and politicians, the Association of the Scientific, Engineering and Technological Community of Canada (mercifully shortened in popular parlance to SCITEC) was formed. It was established in response to the frustrated complaint of a member of the Senate Special Committee on Science Policy that he could not determine which among the many voices really spoke for science in Canada. It was to become a sort of 'parliament of science', and one of its main purposes was to initiate a dialogue with members of the real Parliament in Ottawa.

Since those palmy days, science policy discussions have fallen upon such bad times that when SCITEC recently held its fifth annual meeting, one parliamentarian summed up the present situation thus:

"Science policy is the biggest non-question in the House of Commons. Nobody even knows how to frame a question. On occasions I have been asked to please ask a question of the science minister. Nobody even knows he's there, and a politician can stand anything except not being noticed."

Max Saltsman, New Democratic Party member, had been invited as a member of a panel held after the annual meeting to initiate between parliamentarians and scientists, just the kind of dialogue both have found lacking in the past. The report of the outgoing association president, Dr M. P. Bachynski, said it was SCITEC's intention "that this become a continuing dialogue which could lead to a more formalised vehicle for such interaction —an association of scientists, engineers and parliamentarians."

This hope reflected an earlier one, expressed years ago when the organisation had just got going, that an informal club might be formed for the same purpose. Under the circumstances, the hope seemed rather a forlorn one.

But at least a dialogue had begun. Most of the MPs on the panel, of course, might have been expected to be interested, because they had scientific or engineering backgrounds. They included Harvie Andre, a Progressive Conservative MP with a PhD degree in chemical engineering, formerly an associate professor in that discipline; Frank W. Maine, Liberal MP and former Athlone Fellow, who holds a PhD in organic chemistry and was previously director of research and development in a manufacturing company; and Ross Milne, Liberal, an engineer with a master's degree in environmental control.

Mr Andre was particularly concerned with the present state of public policy relating to science: he called it "a hell of a mess." Recalling the great interest in science policy during the 1960s, he said this had led to an uncomplimentary comparison of Canada's performance with that of other industrialised countries. A whole series of studies had shown that Canada spent proportionately less on research and development than most industrialised nations, and spent less on such activities in industry and more on in-house government research.

The response of the federal government was to create the Ministry of State for Science and Technology (MOSST), but that did not solve the problems, Mr Andre said. "In the four years that department has been in operation, in every way, in each of those [four] areas of comparison, we are worse off." Canada now spends a smaller percentage of its GNP on science and technology; in spite of the MOSST's contracting-out policy, the proportion of in-house research and development has increased rather than decreased (from 65 to 72% of the total); and the percentage of the GNP spent on research and development by industry has about halved.

Mr Andre said he was not implying this was all the MOSST's doing. He felt rather that the ministry lacked muscle to accomplish its objectives. He would like to see it obtain that power—if not, it should be abolished. "It is now a convenient screen to divert attention away from problems and it allows the government to say they are curing the problems."

Dr Virginia Douglas, immediate past president of SCITEC and a panel member representing science, said politicians and scientists both seem to agree that they must get together, yet they also seem to agree that, after endless studies, "we've achieved little communication."

She suggested the trouble was that the two groups work from different assumptions and use different techniques to solve problems. She said the mission-oriented approach is understandably attractive to politicians, but many others are not convinced that a direct, simple-minded approach will solve society's problems. Scientists are being asked to provide simple answers for complex questions—sometimes insoluble ones. And not everything can be subjected usefully to cost-benefit analysis, as politicians would like.

The attempt to control science and put it to work through bureaucracy does not seem to be working, she said, and it endangers the productivity of science. She pleaded with the politicians not to put scientists into a bureaucratic strait-jacket and not to put them into the position of being adversaries. And she said the time has come to stop talking abstractly about policies and start adopting a case approach to the solution problems.

Perhaps one of the best indications of the parlous state of relations between SCITEC and the federal government was revealed in Dr Bachynski's report on SCITEC's attempt to obtain approval of a plan to build a house of science and technology (HOST) to bring together the mostly indigent scientific societies centrally in Ottawa.

The MOSST had never answered the proposal officially, Dr Bachynksi said. But unofficially, the indications were tha the ministry would not support it as SCITEC wanted. "The impression I have is that the ministry would be glad to support such a project as long as it didn't cost the government any money."

Money, of course, was exactly what SCITEC and the other societies needed. But, according to Dr Bachynski, the ministry seemed to think that it would be a difficult idea to sell politically, because all kinds of other groups might then want similar favours and the electorate would not see why scientists should be treated any differently from anyone else. A perfectly understandable view, one is tempted to add. □

Clearing house for cancer epidemiology

THE International Agency for Research on Cancer of the World Health Organisation in Lyon is collaborating with the Deutsches Krebsforschungszentrum in Heidelberg in setting up an international clearing house for the exchange of information on epidemiological studies in cancer. Requests are just going out to epidemiologists to complete a standard form describing the on-going projects. It is hoped that in this way an Annual Directory can be compiled and also that participants can be supplied with current information at any time. The sponsors believe that this may, among other things, help cut down the number of occasions on which similar studies cannot be campared because some relatively minor procedure was not carried out in all cases.

Soviet diary

from Vera Rich

THE publication in the United States last week of tapes purporting to reveal the "last hours" of Colonel Vladimir Komarov, who perished aboard Soyuz 1, can hardly be considered an auspicious augury for the forthcoming Soyuz–Apollo programme. The tapes raise the whole problem of credibility and completeness of information exchange between the Soviet and American participants.

According to the tapes, the Soyuz was out of control for some 12 hours before the disaster, with Komarov fighting desperately to retain control (taking time off, however, for a farewell to his wife and child, and a talk with Premier Kosygin). According to the official Soviet releases, the flight proceeded normally until the moment of deployment of the parachutes. The Tass communique (April 25, 1967) stated: "After implementation of all the operations connected with transition to the landing regime, the ship successfully completed the most difficult and responsible braking sector in the dense strata of the atmosphere, and fully decelerated from the initial orbital velocity. However, when the main parachute was opened at an altitude of 7 km, a twisting of the parachute lines, according to preliminary data, caused the ship to descend at a great speed which resulted in V. I. Komarov's death".

An article by Yuri Gagarin, who was present at the control centre during the whole re-entry procedure, said much the same. The flight was perfectly normal, he maintained, until the moment when the parachutes should have opened. He described the shock felt by all in the control centre when this last-minute disaster occurred. Gagarin's article appeared in *Komsomolskaya Pravda*, the newspaper of the Young Communist movement (young, in this context, meaning 18–35 years)

Certainly, a number of Western commentators at the time suggested that the spacecraft had been out of control for some time before re-entry —these commentators, however, seemed motivated by a preconceived idea that, since "Soyuz" means union, Komarov's craft was meant to be a participant in a manned link-up, but that difficulties with his craft led to the cancellation of the (presumed) second launch and his "premature" re-entry after only 18 orbits.

Another mystery is the state of Komarov's health A background article in *Pravda* of April 24, 1967, which had gone to press before the disaster, stated that after the flight of Vostok 4 (for which he was back-up for Popovich), he had developed a heart murmur, but "a good rest, a more sensible everyday regime, self-confidence and the moral support of his comrades did their work", so that a later medical board found nothing wrong with him, and he was able to make a 24-hour orbital flight in October 1964 Before the flight of Soyuz 1, said this article, he had received "the highest rating". Certainly, it would seem unlikely that any space programme would launch a man in less than perfect health—yet some medical reports which seem to have been written for limited circulation among Soviet experts in space medicine seemed to imply that the proximate cause of the disaster was that Komarov lost consciousness at a crucial moment during re-enry, implying either that the parachute system had to be deployed by a manual control (out of key with the whole tenor of the Soviet space programme, which places so much emphasis on automation that the early cosmonauts were virtually no more than human test animals) or else that, being unconscious when disaster struck, he was unable to make some last minute effort to save himself.

● One of the contributions of the Byelorussian SSR in the current five-year plan is an extensive programme of the study and conservation of the flora and fauna of the republic. This included the launching of a new popular science journal *Rodnaya Pryroda* (Our native nature), research projects by the various scientific institutes of the republic, and a special prize for the best coverage in the media of the problems of conservation. The programme was clearly aimed at local loyalties and sentiment, with extensive use of the Byelorussian language (although current Soviet policy favours the "unifying" importance of Russian).

According to reports in *Rodnaya Pryroda*, the programme is making excellent progress, no less than 13 of the 48 scheduled "themes" being completed three years ahead of schedule. In at least one respect, however, all is not well with conservation in Byelorussia—the close season for fishing is not being respected During the spawning season, persons unspecified are "barbarously" fishing for shallow water fish, to the great detriment of the fish reserves of the republic.

To combat this, "all possible forces" are to be mobilised, ranging from the Fisheries Board of the Council of Ministers of the Byelorussian SSR, through the Byelorussian Conservation Society and the Society of Hunters and Fisherman, down to school-children and the "pioneers" youth organisation. How, precisely, they are to combat the poachers is not explained—presumably by some attempt at patrolling the numerous lakes and rivers of the aquiferous republic. What is more puzzling is who is doing the poaching at all. Byelorussia is not rich in natural resources, and has a long peasant tradition of cautious preservation of the little that is available. Although the new great oil refineries at Novo-Polotsk and Mozyr have brought in many workers from all parts of the Soviet Union, it would be too naive a solution to attribute the depredations to oilmen having a quiet day's angling. The most probable answer seems to be that of 'deculturalisation'—that state planning and state control have destroyed the old, careful traditions, and that rules of conservation which were once an integral part of local tradition now have to be reimposed, by official decree.

● The idea of recovering gold from the sea is not a new one. But attempts to date have all proved uneconomical—the value of the metal recovered being less than the cost of extraction. Now, however, data from the recent Atlantic voyage of the research vessel Mikhail Lomonosov have reopened the question of extracting gold from seawater. Analysis of deep-water samples indicate a gold concentration some 1,000 times higher than expected—a value which, if confirmed for a sufficient number of sites, might well indicate that the metal could be extracted commercially.

The reasons for these high concentrations are as yet a mystery and, not surprisingly, the sites of these anomalies have not been revealed.

● A new means of producing extra-strong concrete has been developed at Naovi (Uzbek SSR)—dropping it This, according to the inventor, Candidate of Technical Sciences A. K. Brovtsyn, produces "complex physico-technical interactions between the grains of the filler and the cement particles", giving an increase in strength of up to 15% A pilot plant for dropping concrete components of up to 3 tonnes in weight on to an elastic base from a height of 35 cm has been developed at a cost of 2,000 roubles (£1,000).

● An artificial method of producing triplets from single-yoked eggs has been demonstrated by embryologist, German Svyatogor of Leningrad University. The method consists of surgically opening the egg, dividing the blastocyst with a micro-scalpel, and then resealing the egg. So far, the Novosti agency reports, triplets have been successfully produced, from the eggs of hens, ducks, turkeys and Japanese quail

correspondence

LEPRA'S money

SIR,—In writing on leprosy (March 20), Dr Crawford claims that the numbers on treatment have remained low for the reasons he mentions and not for lack of money, as LEPRA claims.

The cost of maintaining patients in institutions is enormously high and LEPRA recognised this in 1963 when it carried out a reappraisal of its work. Consequently it has not for many years given grants for maintaining patients unnecessarily in institutions, but still maintains that the numbers on treatment have remained low because of a lack of money, as the cost of finding patients and ensuring their regular treatment on a domiciliary basis for at least three years is very high.

Dr Crawford concludes his article with an accurate example of how LEPRA is using a very small proportion of the money given to it, for a specific purpose. His example, however, may have given your readers an inaccurate picture since the example suggests that voluntary agencies in Europe are still spending most of their income on institutional care This is not the case with LEPRA since its contribution to the fight against leprosy in 1974 was:

- £210,000 on leprosy control projects, mainly for domiciliary treatment.
- £66,000 on grants to encourage early diagnosis for domiciliary treatment of children suffering from leprosy.
- £10,500 on leprosy research.
- £6,600 on training.
- £6,500 on the leprosy aspect of multipurpose rural health projects.

G. F. HARRIS

LEPRA, Colchester, UK

HTRs, zink and aluminium

SIR,—The high temperature gas-cooled reactor (HTR) has frequently been suggested as a source of process heat for steel-making, but we suggest that it could also be used in the zinc —and aluminium—making processes. The key step is the reduction of zinc oxide using a gas heated by an HTR.

At present there are two principal techniques involved in the zinc-making process: chemical reduction using coal, and electrolysis of zinc oxide. The first is carried out at temperatures of 1,200 – 1,400 °C and needs a correspondingly large supply of coke to provide these high temperatures. Zinc oxide might, however, conveniently be reduced using methane or hydrogen with an HTR as the source of process heat. In this case temperatures much below 1,000 °C are necessary—and these can be delivered by today's HTR technology. Natural gas or methane produced by coal gasification using HTR process heat can then be heated by the HTR to 800 °C and can be used for the reduction of zinc oxide. If this reduction has to be done with hydrogen, the latter might be produced by steam reforming of methane or water splitting, using process heat.

The zinc metal produced can either be sold or used in the aluminium-making process. Aluminium is at present produced by molten-salt electrolysis of alumina but high electrical energy requirements and high investment costs are disadvantages of this method. A new purely chemical reduction method, the so-called Toth process, seems to avoid these drawbacks. It is based on a four-step process in which aluminium metal is obtained from an ore containing alumina by way of aluminium chloride. Aluminium chloride is reduced with manganese metal yielding an aluminium–manganese alloy and manganese chloride, which is subsequently oxidised with air and finally reduced with coke at 1,750 °C.

Zinc might be used instead as reducing metal. In contrast to manganese oxide, the zinc oxide formed can be reduced by hydrogen or methane at temperatures far below 1,000 °C.

S. HUWYLER & W. SEIFRITZ

Würenlingen, Switzerland

Nitrogen fixation

SIR,—Does A. B. Lovins (May 1) really believe that those working on nitrogen fixation have not tried to imagine what would happen if they succeeded "beyond one's wildest dreams"?

What if one did develop universal nitrogen-fixing systems for plant life on this planet? The effect would be the same as if adequate conventional nitrogenous fertiliser were universally available: light, water, nutrients other than N (K, P, S, Co, Mo and so on) would become limiting, depending on the particular environments. Assuming all these deficiencies could be corrected artificially, CO_2 fixation would be the final limiting factor—as it is in advanced agricultural industry already.

The ecological and environmental effects would be just about the same as the use of nitrogenous fertiliser has entrained already, but the drain on the world's energy resources (for fertiliser production) would certainly be very much less. The pollution problem arising from run-off might also be lessened, though not eliminated—rotting legumes produce a run-off problem even today. In compensation, a few hundred million people on this planet would be better nourished and a few hundred thousand would be saved from death by starvation each year They would, of course, increase and multiply, so a global problem would be augmented and its solution postponed rather than solved. Yet some feel that there are better ways of tackling the population problem than by accepting starvation.

JOHN POSTGATE

University of Sussex, UK

Ohta and evolution

SIR,—The paper by Ohta (November 29) on which my comments (April 3) have so displeased Nigel Calder (May 1), appeared under the rubric of "Review Article", which might lead the uninitiated (including perhaps Mr Calder) to expect a more or less scholarly summary of recent evidence and theories. What Ohta gave us, in fact, was a highly partisan presentation of the case for one side only on a controversial issue.

My remarks on statistics were perhaps open to misunderstanding; it was not intended to imply that statistical methods have no place in evolutionary studies—for example the statistical work of R. A. Fisher was a very important contribution to the theory of natural selection—but merely to point out that, in the macroscopic world at least, the appeal to chance is essentially an argument from ignorance, and should not be made until causal explanations have been clearly shown to fail

Mr Calder's reference to my remarks on scientific truths makes it clear that there are philosophical differences between us which would need more space for their discussion than this letter could afford. To quote a writer for whom Nigel Calder might have more respect than for the scriptures, William Blake wrote that "establishment of truth depends on destruction of falsehood continually"—in that spirit I was moved to express my criticism rather more forcefully than is usual in these columns.

R. A. CROWSON

University of Glasgow, UK

news and views

Sunspots and the solar cycle

from T. G. Cowling

It is generally agreed that sunspots are surface phenomena, and that their darkness is due primarily to their possessing strong magnetic fields. It is also agreed that bipolar sunspot pairs arise when a bundle of the field lines of a subsurface azimuthal magnetic field has broken through the Sun's visible surface to form a magnetic arch above the surface. Nearly every other assertion made about sunspots has recently been challenged.

In recent years the origin of solar magnetic fields has customarily been explained in terms of dynamo theory. This theory, originating as a modification of a similar theory for the Earth, begins by assuming a poloidal field (one in meridian planes) which is twisted by non-uniform rotation to yield a much stronger toroidal (azimuthal) field. The dynamo has to be completed by a feedback mechanism converting toroidal field back into poloidal; the reconversion cannot be effected by axisymmetrical motions. Parker[1] in 1955 was the first to suggest a suitable mechanism. He showed that the reconversion could be effected by twisting (by Coriolis forces) the field embedded in material being carried upward because of thermal convection in the Sun's outer layers.

Parker's original discussion rested on general physical principles rather than on detailed mathematical analysis. But any defect in this respect has been amply rectified by later workers like Braganskii[2], Steenbeck and Krause[3], and Moffatt[4], who have also suggested other ways of securing feedback. In particular, the mean-field electrodynamics of Steenbeck and Krause, with their isolation of the so-called α-effect (currents parallel to the mean magnetic field) has supplied both a justification for Parker's ideas and a genuinely useful mathematical structure for use in dynamo theory. This structure has been used with conspicuous success in mathematical work on the Earth's dynamo.

In certain respects solar dynamo theory has been similarly successful. For example, as Parker originally surmised, it gives a field progressing towards the Sun's equator, and reversing periodically; this agrees with the observed progression of the sunspot belt towards the equator in any 11-year cycle, and the reversal of polarities in a bipolar spot pair in the next cycle. There is also some observational evidence of a reversing dipole field, at least in high latitudes. This field is, however, weak and uncertain; patches of both polarities are regularly present in each polar cap, and only in an average sense can one speak of the polarity of the cap as a whole. Surface observations provide little evidence of the regular dipole field required to initiate the dynamo cycle.

The observed field is represented more closely by a model suggested by Leighton[5] in 1964, and given mathematical form by him in 1969. This makes use of the tendency of the following spot of a pair to appear at a rather higher latitude than the leader spot, together with the spreading of fields over the Sun's surface by a random walk; this mechanism is employed as a substitute for Parker's feedback process depending on Coriolis forces. Leighton was able to get a 'butterfly' diagram in good agreement with observation for the progression of the sunspot zones towards the equator in any cycle; dynamo theory based on the α-effect makes new spots of a cycle appear at excessively high latitudes. Leighton also found a reversal of the polar (dipole) fields two or three years after the beginning of a new cycle, as observed, whereas α-effect dynamos tend to make the two nearly simultaneous.

Part of Leighton's success was due to his introduction of 'threshold' terms effectively limiting field generation to low latitudes, and ensuring that high-latitude fields arise only through the random walk. Apart from these threshold effects, Leighton's equations closely resemble those of an α-effect dynamo and, as Stix[6] recently showed, the α-effect dynamos can mimic Leighton's results if the law of non-uniform rotation is chosen such as to confine field generation to low latitudes. Leighton's discussion is in any case subject to the criticism that it relies over-heavily on purely surface observations, and gives little insight into the physics of the dynamo process at greater depths. Since the latitude effect invoked by Leighton is itself likely to arise from Coriolis forces, it is reasonable in discussing physical processes to confine attention to α-effect dynamos resembling that of Parker.

Piddington[7] has criticised such dynamos, on two main grounds. The first is that they create their new poloidal flux always at a higher level than the original flux, so that ultimately they should push the magnetic field out of the Sun. The second is that in order to get field reversal one needs not only to create new flux but to destroy unwanted old flux, and the electrical conductivity of solar material is too great to permit destruction in the relatively short times allowed. The reply of the dynamo theorists[8,6] has been that ohmic dissipation is supplemented by turbulent diffusion, which brings oppositely directed fields into close juxtaposition, so facilitating their mutual annihilation. Turbulent diffusion is also regarded as able to bring new magnetic flux down to the level of the old.

Piddington refuses to accept this reply as valid. He points out that surface observations do not support the concept of violently tangled fields, but show large patches of one polarity existing side by side with similar patches of the opposite polarity; small-scale dissipation, such as Stix[6] suggests on the basis of solar 'filigree' observations, can destroy small irregularities within the polarity patches but not destroy the patches themselves. He also argues that the concept of turbulent diffusion, though useful in discussing the transport of scalar quantities, is less satisfactory when discussing magnetic problems, where field lines have to be transported across field lines not in the same direction. Parker[8] has discussed mechanisms for speeding up the relinking of field lines in such transport. It is uncertain whether these mechanisms can work below the Sun's surface; Piddington asserts that in any case they cannot work fast enough until the field has grown so strong as to be able to choke the convective motions supposed to generate it.

Thus, though the dynamo equations certainly do give solutions that agree with the observed fields, Piddington asserts that these equations do not represent physical reality. Instead of the relatively diffuse fields of dynamo theory, he regards solar fields as composed of twisted 'ropes' (bundles of field lines) whose fields are strong enough to defy permanent distortion, and which are nearly indestructible. He quotes observational evidence in favour of this point of view. Away from sunspots, most of the surface magnetic flux is concentrated in small elements, with fields approaching 1,000*G*, situated at the junctions of supergranular convection cells. On dynamo theory such fields are supposed to be concentrated by the convection; he believes that they control it. More convincing evidence is provided by the facts that, when magnetic fields burst through the Sun's surface, they appear to do so as sets of individual ropes[9]; when a spot decays, it does so by peeling off ropes which are seen as magnetic 'knots' moving across a 'moat' surrounding the spot.[10]

Piddington envisages a sunspot magnetic field as a stranded cable of magnetic ropes. He regards twists of the ropes as essential, to avoid an exchange instability that would otherwise explode the field (however, this instability is not yet definitely established). He is unable to get a reversal of the Sun's field as a whole, and to this extent his ideas compare unsatisfactorily with those of dynamo theory. The best he can do is to create a reversing toroidal field from a nonreversing poloidal field limited to the deep interior, by a torsional oscillation similar to that originally considered by Walén[11] in 1948. As Stix[6] has emphasised, this idea meets with a number of difficulties, not the least being that it postulates a subsurface field unrelated to anything that we actually observe.

But the dynamo theory is subject to a similar objection. The polar field observed on the Sun is not the regular poloidal field posited by dynamo theory as its starting point; it seems rather to be an epiphenomenon, left over from sunspot fields. Also, as Altschuler *et al.* have shown[12], and as is confirmed by observations of the sector structure of interplanetary fields[13], the dominant large-scale field of the Sun rarely is that of an axial dipole; more often it is that of an equatorial dipole or quadrupole Thus, though the remarkable success of dynamo theory as a mathematical theory must be acknowledged, doubts must persist whether it explains the actual solar fields. One cannot but wish that one could look beyond the Sun's outermost atmosphere, and see what is going on at greater depths.

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Drugs and nucleic acids

from Stephen Neidle

The nucleic acids DNA and RNA are undoubtedly among the best defined of all target molecules for the action of drugs, antibiotics and anti-bacterial agents. This has encouraged very many attempts over the past twenty years to define the molecular geometries of such drug–nucleic acid interactions, often with the ultimate hope of more effective and rational drug design.

It is nearly fifteen years since Lerman proposed his intercalation model for the interaction of aminoacridine mutagens, such as proflavine, with DNA; this model has survived the test of time rather well, at least in general terms. Lerman proposed that the planar drug molecule could slot (that is, intercalate) between the base pairs of DNA, with consequent unwinding of the double helix. He and numerous other workers have demonstrated by a variety of physical methods that many of the changes in DNA properties observed on binding can be directly attributed to intercalation. Unfortunately, although it has been established that helical unwinding does occur, the precise degree of unwinding has long been a point of uncertainty. Estimates have varied from 12° to 45° per drug insertion. Only very recently has the matter been clarified; the precise studies of Wang, using a buoyant density gradient caesium chloride titration method, have indicated that the correct value is 26° (*J. molec. Biol.*, **89**, 783; 1974). Although Wang's studies were on the DNA–ethidium system, there is good reason to believe that many other intercalating drugs untwist the helix by a similar amount.

Support for the intercalation model has now come from the crystallographic analyses of Sobell and his coworkers. They have capitalised on several recent findings that self-complementary dinucleoside monophosphates, such as adenylyl-(3′, 5′)-uridine, form Watson–Crick hydrogen-bonded dimers in the solid state, which can be considered to be RNA double-helical fragments. Sobell *et al.* have therefore used such fragments as nucleic acid models, and they have complexed them with the trypanosidal drug ethidium bromide, a well established intercalating agent. The preparation of complexes with 5-iodouridylyl-(3′, 5′)-adenosine and cytidylyl-(3′, 5′)-guanosine was briefly reported at a recent Royal Society Discussion Meeting, and Tsai, Jain and Sobell have now published an account of their X-ray structural studies on the former (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 628; 1975). The structural unit consists of a Watson–Crick base-paired dimer, to which is attached two ethidium ions. One of these is positioned between the base pairs of the now opened-up miniature double helix, and the other stacks externally on top of them. The intercalated ethidium has unwound the dinucleoside phosphate by about 12° compared with its uncomplexed conformation—this corresponds to unwinding angles of 26° for RNA, and 29° for DNA, assuming a direct correlation between model and polymer (nucleic acid) intercalation. As the authors do indeed point out, this assumption is unproven, in spite of the satisfying agreement of their result with Wang's.

There are in fact several features of this intercalative complex structure that may well belong merely to very small nucleic acid fragments, as well as to this particular analysis. Such a feature is the unexpected mixture of nucleotide sugar conformations, which could well have a significant effect on the unwinding angle. This structure forms an interesting contrast to that recently reported for another dinucleoside monophosphate–drug complex (Seeman, Day and Rich, *Nature*, **253**, 324; 1975), between the mutagen 9-aminoacridine and adenylyl-(3′, 5′)-uridine; this complex has an open, non-double-helical structure with drug molecules stacking on either side of, this time, Hoogsteen-type adenine–uracil hydrogen bonds in contrast to Watson–Crick base-paired adenylyl-(3′, 5′)-uridine itself. It is likely that the complex interplay of intramolecular forces which serves to stabilise double-helical nucleic acids is finely balanced for dinucleoside monophosphates. There seems to be no guarantee that these can preserve their 'mini nucleic acid' features when complexed with drugs. Probably only rather longer complementary oligonucleotides will satisfactorily reveal the molecular features of drug intercalation, especially subtle cooperativity and nearest-neighbour effects.

Prominent among many putative intercalating drugs is the plant alkaloid ellipticine, which has a planar ring structure capable of being inserted between base pairs. Ellipticine has received widespread attention on account of its high anti-leukaemia activity, which is believed to be related to its binding to nucleic acids (compare other anti-cancer drugs, such as actinomycin and daunomycin). Kohn, Waring and their co-workers have now established that this drug does indeed bind to DNA by an intercalative process (*Cancer Res.*, **35**, 71; 1975). The unwinding angle is less than that for ethidium, but very close to that for proflavine, another classical intercalating agent. Indeed ellipticine and proflavine have generally very similar binding behaviour, at least with calf thymus DNA, presumably because their molecular dimensions are so similar.

Le Pecq, Xuong, Gosse and Paoletti have used the intercalating properties of ellipticine in a systematic attempt to obtain highly active anti-cancer drugs by consideration of various ellipticine molecular modifications (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 5078; 1974). Their very simple reasoning was that, assuming DNA is the genuine cellular receptor for these drugs, a high degree of binding could correlate with high anti-tumour activity. DNA-binding constants and unwinding angles, and activity against L1210 mouse leukaemia, were determined for a series of derivatives; 9-hydroxyellipticine had the highest DNA affinity, and moreover had the strongest pharmacological activity. It seems plausible that the introduction of the hydroxy group has enhanced binding, by means of a hydrogen bond to a DNA phosphate group.

Of course, as Le Pecq *et al.* point out, strong binding to DNA does not by itself confer anti-tumour activity, as opposed to other biological activity— one of the derivatives tested by Le Pecq *et al.* had marked trypanocidal properties only. Many other molecular and indeed cellular properties are important. But one can certainly look forward to other successful applications of this straightforward approach to drug design.

A new theory of the Universe

from P. C. W. Davies

Fred Hoyle has long been known as a man with provocative and radical ideas about the nature of the Universe. During the 1950s and early 1960s he championed the famous steady-state theory of cosmology, in which the gross features of the Universe were assumed to remain unchanged for all eternity, a situation to be maintained by the continual creation of matter to fill the gaps left as the Universe expands.

This theory proved highly attractive philosophically, and was for a time very popular among cosmologists, but towards the end of the last decade, mounting observational evidence seemed to indicate that, rather than remaining unchanged, the Universe had a birth, growth and death, after the fashion of a biological organism. The 'big bang' theory is based on the assumption that the Universe was created about 15,000 million years ago in a hot, dense and violent expansion. A faint glow from this initial fireball is still detectable today, in the form of thermal radiation bathing the Universe at a feeble three degrees above absolute zero. This radiation is known in the trade as the microwave background.

Latterly, Professor Hoyle has accepted the concept of a hot big bang, but in a recent paper in *The Astrophysical Journal*, he describes a new and somewhat unusual perspective on this momentous event. In order to understand this departure, it is necessary to know something of the theoretical background to cosmology which Hoyle has developed with his co-worker Jayant Narlikar.

A perennial headache among physical scientists is to know which system of units to adopt for measurement of physical quantities. As it happens, nature supplies us with certain universal quantities against which it appears natural to measure everything else. Two such quantities are Planck's constant and the speed of light. Accepting these as our basic units of measurement, all other physical quantities may then be measured in terms of mass. For example, the mass of the electron is associated with a length of about 10^{-13} cm and a time interval of about 10^{-23} s.

Now it is generally accepted that the Universe is in a state of expansion, which is best visualised as a systematic and uniform growth in the scale of cosmic distances. To be specific, every day the observable Universe grows in scale by 10^{16} cm, or about 10^{29} electron mass units. This steady growth implies that in the past the Universe was shrunken, and simple versions of the big bang theory demand that 15,000 million years ago the shrinkage was complete, with the whole of the Universe which we at present observe squeezed up into a single point. This awesome event is generally known as a singularity, and is usually identified with the creation of the Universe. Certainly physical reasoning cannot be extended back through such a singularity.

The novel proposal made by Hoyle is to turn the cosmic scale change around, and regard the mass as the quantity which varies while the Universe remains static. Such a situation can be procured (mathematically) by changing the scale of measurement at different moments of time, something known to mathematicians as a conformal transformation. By thus transforming away the cosmological motion "The usual mysteries concerning the so-called origin of the Universe begin now to dissolve", according to Hoyle.

Lest the reader be suspicious about the creation of the Universe being removed at a (merely mathematical) stroke, even from the pen of so auspicious an individual as Professor Hoyle, something of the theoretical framework surrounding this new perspective should be mentioned. The use of conformal transformations to remodel the geometry of the Universe is often used for convenience of mathematical discussion. In this way the violent shrinkage of space near the big bang can be

A hundred years ago

The new Reptile House in the Jardin des Plantes, Paris, has sustained some heavy losses. A large turtle died from the shot it had received many months ago when captured in the Atlantic Ocean, and a large serpent from a wound inflicted by a rat. The rat having been offered as living food, resisted violently, and bit his adversary so deeply that he died a few days afterwards. The warders in the picturesque Reptile House will probably be more cautious in future in showing visitors the spectacle of Ophidians running after their food.

We are glad to say, however, that the above heavy loss will be to a considerable extent compensated, as the Jardin des Plantes will receive in a very few days a Boa more than eight yards in length, which has just arrived at Havre. We believe it takes a goat or a sheep to appease its appetite at one time.

from *Nature*, **12**, 54; May 20, 1875

'stretched out' again. The physical relevance of these transformations is usually limited, though, to considerations of systems without mass (for example, electromagnetism), simply because a non-zero mass changes under the transformation, and this is something not normally encompassed by conventional theories. But Hoyle appeals to a theory of mass and gravitation which he developed some years ago with Narlikar. In this theory, the mass of a particle is not a fixed, intrinsic attribute, but arises by an interaction between the particle and a 'mass field' which is in turn produced by all the other matter in the Universe, in a fashion often associated with the ideas of Mach. As a result, the mass field (and hence the particle mass) may vary from place to place, and time to time. By thus making variable mass a foundation stone of the theory, the use of conformal transformations fits quite naturally into the scheme. Not only massless systems, but all of physics, now become invariant under these transformations.

The utility of this extra flexibility is that the physicist is free to choose which conformal frame to work in. For example, by choosing the masses to be constant, the usual picture of an expanding Universe is obtained, and indeed the Hoyle–Narlikar theory then reduces to the conventional general theory of relativity. The singularity which occurs at the beginning of the big bang in this picture cannot be avoided in general relativity as a real physical catastrophe. But in the Hoyle–Narlikar theory this event merely signals a mathematical breakdown in the fixed mass frame, which may be neatly sidestepped by conformally transforming to the fixed Universe–varying mass frame.

The 'creation of the Universe' is then relegated simply to a moment when all masses happen to vanish, something which occurs quite naturally from time to time, according to Hoyle. The reason for this is that there exist both positive and negative sources of the mass field (reminiscent of positive and negative charges in electrodynamics), so that now and again an exact cancellation may occur.

Having thus argued that the big bang was not a creation event at all, Hoyle then goes on to ask the question: what happened before? In a brief discussion, some of the features which we now observe in the Universe and attribute to unknown properties of the big bang, are connected instead with processes occurring in an earlier phase. Some of the suggestions are not new, such as the energy to heat the fireball (visible now as the microwave background) originating from the accumulated starlight of an earlier phase. Others, such as the preservation of some residual stellar structure through the big bang/mass vanishing phase are quite unusual. The latter is used as an explanation of the deuterium to hydrogen ratio in the Universe, by speculating that synthesis of deuterium took place in regions of much lower density than average.

In summary, Hoyle has produced a picture of the cosmos which bears some similarity to the steady-state theory (no creation event) but still incorporates the essential features of the big bang fireball. The vital ingredient of the steady-state theory—the continual creation of matter to maintain constant entropy conditions—is not present, however. Consequently the new model suffers from the usual paradox of all infinite age cosmologies: why haven't irreversible processes generated unlimited entropy? Shouldn't the accumulated starlight in the fireball be arbitrarily hot from an infinity of previous phases? Nevertheless, many physicists may find this imaginative new picture a comforting circumvention of the unpleasantness of the initial singularity.

Disagreements over Troodos

from Peter J. Smith

DURING the past decade or so, almost all earth scientists with experience of the Troodos Massif of Cyprus have managed to convince themselves (and most others) that this ophiolite sequence is an upthrust fragment of ancient ocean floor and thus originally formed at an ocean ridge. It is a remarkable achievement, not least because the evidence, though strong and diverse, is almost entirely circumstantial; and the data required to remove all shadow of doubt remain to be found. In 1973, however, the consensus which had taken so long to build up was abruptly shattered by Miyashiro (*Earth planet. Sci. Lett.*, **19**, 218; 1973) who proposed instead that Troodos was created as a volcano in an island arc between Eurasia and Africa. The verbal reaction to this unexpected heresy was immediate, but the more temperate and reasoned criticisms have only now begun to appear in print.

Miyashiro based his case largely on comparisons between the geochemistry of Troodos rocks and that of recent ocean floor, on the one hand, and between Troodos geochemistry and island arc geochemistry on the other. In particular, he used SiO_2 contents and Fe/Mg ratios to show that the lower pillow lavas and the sheeted complex of Troodos contain a relatively high proportion of rocks with calc-alkalic affinities as well as others which follow the more expected tholeiitic trends. The significance of this discovery is that, if dredge samples are anything to go by, calc-alkaline igneous rocks of deep oceanic origin seem to be very rare. By contrast, island arc volcanism and calc-alkaline rocks are frequently observed in association. The implication, therefore, is that Troodos (and possibly other ophiolite sequences) is more likely to have been formed in an island arc environment than along an oceanic ridge.

The first serious criticism of this thesis now comes from Hynes (*Earth planet. Sci Lett.*, **25**, 213; 1975) who argues that rocks of the Troodos complex are so altered that bulk chemical analyses must be regarded as suspect, if not actually worthless, as indicators of origin. Indeed, the extensive alteration of the sheeted complex and the lower pillow lavas has been recorded by many workers since at least 1959 (see, for example, Wilson, *Geol. Surv. Dep. Cyprus Mem. 1*, 1959) and the abundance of secondary silica in Troodos rocks has been a frequent theme. On these grounds, therefore, any direct comparison between the chemistry of Troodos and that of unaltered rock from the ocean floor cannot be valid. To illustrate the dangers of placing too much reliance on chemical data, Hynes goes on to describe the Othris ophiolite suite in Greece which comprises rocks lithologically and petrographically similar to those of Troodos. The chemical characteristics are similar too, which would imply that Miyashiro's arguments are equally applicable. But in the Othris case there is stronger mineralogical, and especially regional geological, evidence in favour of a ridge origin against which the chemical data pale into relative insignificance.

In his reply to Hynes, Miyashiro (*Earth planet. Sci. Lett.*, **25**, 217; 1975) admits that some Troodos rocks have been subjected to secondary alteration but claims that these lie mainly in the upper pillow lava series which he ignored for that very reason. By contrast, rocks in other parts of the complex are "not so unusual in chemical composition". Many such rocks, for example, contain 70–80% anhydrous SiO_2; and if this had been brought about by the metasomatic introduction of silica into a basalt containing only 50% SiO_2, the proportion of material added from outside would have amounted to more than 100% of the original basalt. This, claims Miyashiro, is "not plausible". Primary silicic igneous rocks do therefore exist in Troodos and useful information may be obtained from their bulk chemical compositions.

In brief, this information is that about half of the volcanic rocks in the sheeted complex and lower pillow lavas of Troodos have $SiO_2>52.5\%$ and $FeO^*/MgO>2.0$ whereas most ocean ridge volcanics have lower values for these quantities. This is "decisive" evidence against an oceanic origin for Troodos but is consistent with formation in an island arc (although it is also consistent with a continental origin). Moreover, Miyashiro says that he "cannot understand" why the geological information quoted by Hynes supports the existence of accreting plate margins in the vicinity of Mesozoic Greece, arguing instead that this evidence is "so ambiguous" that it could be taken to support either view.

More generally, Miyashiro suggests that the world's ophiolite sequences are so diverse that some were probably created in island arcs, others at oceanic ridges and yet others in hot spots and stable continents. This point is well taken by Moores (*Earth planet. Sci. Lett.*, **25**, 223; 1975) who nevertheless feels that Miyashiro is wrong about the particular example of the Troodos Massif. Moores notes, for example, that much of Miyashiro's case is based on the premise that calc-alkalic rocks are rare in the ocean floor. But the fact is that most of the rocks obtained from the deep ocean floor have been dredge samples which are predominantly surficial and thus not representative of the oceanic crust as a whole. In this respect, Miyashiro has not been comparing like with like. When a crustal cross section has been sampled (for example, on faults in the Mid-Atlantic ridge and the Ninety East ridge), the rocks have been found to have compositions comparable to those of ophiolite sequences.

On the question of alteration, Moores has re-examined sections of the Troodos rocks analysed by Moores and Vine (*Trans. R. Soc.*, **A268**, 443; 1971) and finds that almost all of the rocks are indeed altered, most of them having abundant vesicles filled with zeolites or silica and many representing greenschist facies metamorphic assemblages. Thus in spite of claims to the contrary, Miyashiro has apparently been citing data influenced by metasomatic alteration. Primary SiO_2-rich igneous rocks do exist in ophiolites; but they account only for a very small proportion of the total volume and are significantly different from the genuine calc-alkilic rocks found in island arcs, especially insofar as they have much lower K/Na ratios. Moores thus concludes that bulk chemistry is not an adequate basis for differentiating between the products of ridges and island arcs and that recourse must be made instead to structural and stratigraphic arguments.

In a much longer reply to Moores, Miyashiro (*Earth planet. Sci. Lett.*, **25**, 227; 1975) attempts to refute his opponents' arguments in both general and specific terms,' on the basis both of "logic and evidence". On the question of ocean floor sampling, he claims (with evidence) that the Ninety East ridge is atypical of oceanic ridges and that there are no grounds for assuming that the mid-Atlantic ridge fault represents a particularly deep section of oceanic crust. At the same time, sampling is not as unrepresentative as is sometimes claimed because rocks formed at depth are exposed along median valley walls and in transverse fracture zones across oceanic ridges. On the question of alteration, he presents additional data to support the view that Troodos compositons cannot be attributed to weathering, metamorphism or metasomatism but arise in-

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Anions and membranes

from a Correspondent

THE identification, isolation and partial characterisation of a human erythrocyte membrane protein essential for anion transport (Cabantchik and Rothstein, *J. Membrane Biol.*, **15**, 207–226 and 227–248; 1974; Ho and Guidotti, *J. biol. Chem*, **250**, 675–683; 1975) provides strong support for Gunn's hypothesis that the various anion exchanges observed in the red cell occur through the passive diffusion across the membrane of a common carrier–anion complex. It is of interest to compare this work with the recent work, particularly that of Narumi's group (*Biochim. biophys. Acta*, **311**, 80–89 and 90–97; 1973), on the mechanism of HCl secretion by gastric mucosal cells. As part of this mechanism internal bicarbonate is exchanged for external chloride, a situation analogous to that in the red cell, but in contrast to the red cell there is evidence of an active bicarbonate transporting system.

For many years it was thought that simple anions crossed the red cell membrane, diffusively, as free ions and that the differential permeability of the membrane to cations and anions was the result of fixed positive charges within the membrane. This theory was particularly attractive in the light of Bangham's work showing that the permeability of a simple lipid bilayer to different ions could be altered by changing the surface charge of the bilayer. A less attractive feature of the theory was the high activation energy of ion transfer.

This activation energy was closer to those usually associated with active ion transport. When it was shown that the rate of transport of several anions reached a saturation level as the ion concentration was increased the free ion diffusion theory had to be replaced by one involving a carrier ion complex Cabantchik and Rothstein showed that the carrier was a glycoprotein by treating red cells with a non-penetrating fluorescent marker (SITS). and demonstrating that sulphate permeability was inhibited when a relatively small population of sites on the external side of the membrane were labelled. They then isolated the label by proteolytic digestion and showed it to be attached to a glycoprotein of molecular weight roughly 9,500 Ho and Guidotti have also isolated a glycoprotein of similar size which is responsible for phosphate transport and identified it with Bretscher's component a. It is tempting to assume that these proteins are identical and are also responsible for the physiologically important chloride–bicarbonate exchange but this has yet to be shown.

As the mechanism of anion exchange in the red cell has become clearer that of the gastric mucosal cells has become more complex. The presence of a bicarbonate ATPase in gastric mucosa, and several other tissues where bicarbonate transport is important, has suggested the presence of a bicarbonate transporting mechanism analogous to the ATP-dependent cation pumps The demonstration by Namuri and Maki that carbonic anhydrase, which is necessary as a source of bicarbonate ions in gastric mucosal cells, is activated on phosphorylation by a cyclic AMP-dependent kinase would seem to complete the picture by linking the bicarbonate pump to hormones which control gastric secretion and which are thought to act by increasing the level of cyclic AMP in the acid-producing cells. The major problem in this picture is the localisation of the ATPase most of it is found in the mitochondrial and not the plasma membrane fraction (Soumarmon, Lewin, Cheret and Bonfils, *Biochim. biophys. Acta*, **339**, 403–414; 1974). But a bicarbonate pump in the mitochondrion would itself be of interest and the isolation of this ATPase as a Triton extract may indicate that a comparison between a passive and active carrier for the same ion will soon be possible.

stead from crystallisation differentiation. Moreover, he suggests that, by involving silica metasomation for Troodos, supporters of the ridge origin are contradicting themselves insofar as modern oceanic ridges show no signs of metasomatism.

In a final critical article, Gass *et al.* (*Earth planet. Sci. Lett.*, **25**, 236; 1975) point out, among other things, that in discussing the FeO*/MgO ratio Miyashiro has taken no account of the replacement of Mg-bearing mineral phases by carbonates and other non-magnesian pseudomorphs, and that the analyses quoted by Miyashiro were carried out before the precise boundary between the upper and lower pillow lavas was defined (so that many are in fact from the upper lavas). To these specific objections, however, Gass and his colleagues add what is perhaps the most telling general point of all, namely that in this whole argument there has been more than a tendency to ignore the wide variety of evidence available.

The fact is that few, if any, workers have claimed that an oceanic origin for Troodos has been proved. Instead, the case is based on an extremely wide range of evidence from many different branches of the earth sciences, with no single piece of evidence yet obtained being regarded as conclusive by itself. By concentrating public disagreement largely on the geochemical data, support for Troodos as ancient ocean floor has thus somehow been made to appear more equivocal than it really is. For the time being, the balance of argument remains with Miyashiro's opponents.

Rotating fluids

from a Correspondent

'Can the geomagnetic dynamo be powered by precession?' was one of the geophysically motivated questions raised at the recent Euromech meeting entitled "Non-linear processes in rotating fluids" held at University College, London between April 14 and 17.

THE sufficiency of precession as an alternative power source to convection in the Earth's core was discussed by Jacobs (University of Cambridge). Estimates of the power required to maintain the geomagntic dynamo against ohmic dissipation have been variously assessed as between 10^9 and 10^{12} W with the probable value between 10^{10} and 10^{11} W. According to Jacobs and his co-authors (Rochester, Smylie and Chong) the precessional power input to the core (at the expense of the obliquity) fails by at least two orders of magnitude to satisfy this requirement, and thus the possibility of a precession-driven dynamo is extremely remote. This contrasts with the views of Malkus (*Science*, **160**, 259; 1968) and, more recently, Stacey (*Geophys. J.*, **33**, 47; 1973) who have both argued in favour of precessional torques producing the driving mechanism for dynamo action. Jacobs attributed the disagreement not only to Malkus's failure to allow for electrical conductivity contrast at the core–mantle interface and Stacey's incorrect determination of the tilt-over angle, but also to the neglect by both authors of the dependence of the strength of the dissipative part of the core–mantle coupling on the diurnal frequency of the precession-induced core flow relative to the mantle. Loper (Florida State University and University of Newcastle upon Tyne) was equally pessimistic regarding the role of precession. His investigation of the transmission characteristics of the core's hydromagnetic boundary layers indicated again that precessional power is inadequate by at least 10^2.

In attempting to extend further the laboratory modelling of atmospheric flows in the rotating annulus, Leach (Meteorological Office) investigated the particular effects of topography and found that its presence significantly affected the well-known transitions between the different flow regimes and that it reduced the amplitude of the free baroclinic waves. The interplay between these free waves and those generated by the topography resulted in an increase in heat transfer, indicating that the free waves play a diminished part in the energetics of the system. A related study by Mason and Hide (Meteorological Office) into the effects of sloping end walls upon the transition from axisymmetric flow to baroclinic waves was illustrated by an interesting cine film. Various combinations of end-wall orientations were investigated and non-linear effects were identified by comparing the observed wave numbers with those predicted by appropriate linear theory.

The meeting also included contributions from other branches of fluid mechanics. Wimmer (University of Karslruhe) reported a very careful experimental investigation into the viscous flow between rotating concentric spheres (the outer sphere usually being stationary). Measurements of the friction torque were presented for a wide range of gap widths and Reynolds numbers. Photographs of the transitions from laminar stable flow to unstable flow to turbulent flow were shown and it was stated that, in addition to the well known Taylor–Görtler-type vortices, several new types of instability were discovered. One example in the wide gap case was a class of instability confined to the equator (the number of vortices being independent of Reynolds number) and having five different modes of instability for the same Reynolds number.

Several numerical investigations into atmospheric phenomena and related laboratory models were presented, including two papers by Hoskins (University of Reading) and Simmons (University of Reading) on non-linear properties of baroclinic waves and the formation of fronts, and the results of Quinet (Meteorological Institute, Brussels) on a numerical simulation of amplitude and tilted-trough vaccilation observed in the annulus experiments. Egger (University of Munich) reported the results of his numerical experiments on the cyclogenesis occurring in the lee of a long north–south mountain chain as this barrier is approached by a depression. By studying the time-development of the system it was claimed that a satisfactory mechanism for the cyclogenesis process could be obtained.

Space tribology

from a Correspondent

The first symposium in Europe, if not in the world, to be devoted to space tribology was organised by the European Space Research Organisation at its establishment at Frascati on April 9, 10 and 11.

THE papers varied from a very fundamental contribution by M. Barquins, R. Courtel and M. Maugis (Centre National de la Recherche Scientifique, Meudon) on the friction of Cu–Cu, Mo–Mo, Cu–Mo and Cu–Ni couples in ultra high vacuum to a paper by E. J. Robbins (European Space Tribology Laboratory, Risley) on the testing of the tribological performance of space mechanisms in a vacuum, in which the author presented a clear picture of the true environment in the satellite and considered the effect of water and other desorbed vapours on spacecraft mechanisms.

In general the emphasis was upon dry lubricated systems and a whole session was devoted to lead film lubrication of rolling element bearings, which is a largely European development, with now over 10^6 hours of vacuum operation to support it. The limitations of the performance of the film in air were clearly indicated in a paper by M. J. Todd (National Centre for Tribology, Risley) who reported tests showing rapid bearing failure at speeds beyond about 20 r.p.m.

Another session was devoted to the performance of slip rings in ultra high vacuum, mostly operating in the dry lubricated regime. The behaviour of slip rings under these conditions is surprisingly good, both from the point of view of low noise and low wear, and it is now possible to use them for mechanisms requiring lives of 6 to 7 years in space. The four papers in this session show that the European and US approaches are very similar, both using Ag.Cu.MoS_2 compact brushes on silver slip rings. A paper by W. Wilkens (DFVLR, Braunschweig-Flughaven) presented some new data on the performance of dry lubricated slip rings moving at very low speeds, such as are encountered on solar panel drives on communication satellites.

The only paper presenting work on gears in UHV came from E. Conde who reported some of the results of a very extensive programme at the CNES establishment in Toulouse. There seems to be no other work on the performance of gear pairs in UHV in progress in Europe.

One of the most important tribology problems for long life spacecraft is the provision of a bearing–lubricant system for momentum wheels. These units normally rotate at 3,000–4,000 r.p.m. and must perform reliably for 7 to 10 years in space. All momentum wheels flown to date are mounted on ball bearings with controlled minimum lubrication systems. W. Auer of Teldix, Heidelberg, presented a paper on the development of a European momentum wheel which is now flying in the Symphonie satellite and which embodies a lubricant store that progressively releases its oil to the bearings.

A unique and very interesting development in Europe is the application of grease lubricated hydrodynamic bearings to momentum wheels. Philips of Eindhoven have pioneered this work in the world and E. A. Muydermann and U. van der Wal presented two papers which discuss both the principle of the design and the present state of development. Nine bearing systems have each now completed more than 44,000 hours operation under various conditions of load and environment and the authors are now confident that all problems of shear instability and retention of the grease have been solved. The wheel has been chosen to fly in the ESRO Orbital Test Satellite now under construction

Also in the field of liquid lubrication was a paper by G. C. Friend, A. J. Grosiek, W. A. Ware and M Warwick introducing four new space lubricants developed by BP Research Centre Two of these are fine cut oils of very low vapour pressure, one a petroleum oil and the other a di-ester, which are available specially filtered in small quantities. The other two are greases based upon the oils but using oleophilic graphite to perform the dual role of boundary lubricant and thickener These lubricants are now undergoing life tests.

A very important paper by B. Baxter (British Aircraft Corporation, Stevenage) presented the results of a physical-chemical examination of the degradation of a grease which had run in a bearing for some 14,000 hours in vacuum. The author has developed at BAC a technique for examining the interaction of bearing and lubricant, which in this case was able to show that the 12 hydroxy-stearic acid in the grease-thickening agent is lost during running in low oxygen conditions. The technique is extremely valuable in gaining deeper insight into the reasons for bearing–lubricant system failure and the same author employed it very successfully in 1972 to explain the well known but poorly understood formation of 'brown sugar' in miniature bearings. The use of this technique in space tribology is very new but it can be expected to make significant contributions in the future.

The need for some harmonisation of the space tribology work in Europe was stressed by H. M. Briscoe, ESRO, in his closing address. ESRO is partly able to perform this task by acting as a clearing house for information and as adviser on future requirements, but he suggested that a small international advisory committee might be more effective at national level in preventing unnecessary duplication.

Crystallisation and chain extension in polymer solutions

from Paul Calvert

In recent years the study of morphology in crystalline synthetic polymers has reached an impasse. Twenty years ago the discovery that 10 nm, plate-like crystals could be grown from solution led to the development of the folded chain model, by Keller and others. Now, thousands of man-years of research and several hundredweight of publications later there is still little hard evidence as to whether the chains fold tightly or loosely and whether re-entry is adjacent to the point at which the chain leaves the crystal or whether it is at random. One way to try to gain more understanding is to look at the effect of aligning the chain before crystallisation. Mackley and Keller (*Phil. Trans. R. Soc.*, **278**, 29; 1975) describe such a study of molecular orientation and crystallisation in flowing polymer solutions.

In 1965 Pennings and Keil (*Kolloid Z.*, **205**, 160; 1965) described fibrous 'shish kebab' crystals, grown from stirred polymer solutions at high temperatures where unstirred solutions would not crystallise. These structures have been studied in detail by Pennings *et al.* (*Kolloid Z.*, **235**, 99; 1970; **237**, 336; 1970) by Krueger and Yeh (*J. macromol. Sci. (Phys)*, **B6**, 431; 1972) and by others. The shish kebab, of overall diameter about 1 μm and length up to several mm, apparently has a central core, the 'shish', about 20 nm diameter skewering lamellar crystals, the 'kebabs'. The shish and kebab are however molecularly connected, not distinct. It was found that turbulence was necessary for their formation, probably because this resulted in elongational as opposed to shear flow and so straightened the dissolved molecules.

Mackley and Keller give a detailed description of results obtained with an apparatus designed to produce pure extensional flow, first described with Frank (*Polymer*, **12**, 467; 1971). Two opposed jets of solution give extensional flow in the symmetry plane when both blow and on the common axis when both suck.

Calculations for dissolved polymer chains suggest that there should be a rapid increase in molecular extension at a critical flow strain rate, which is molecular weight dependent. Observations on the appearance of reversible birefringence in solutions above their crystallisation temperature support this and suggest that molecular alignment is almost complete. It should be noted though, that 95% alignment as measured by birefringence could still correspond to one sharp bend for every twenty chain units, so that the molecule is locally aligned yet compact in overall shape, like a pack of sausages. It would be helpful to have more information on molecular shape, such as a light scattering study might provide.

As the temperature is lowered fibrous crystallisation takes place in the high strain-rate regions of flow. The maximum temperature for crystallisation increases with strain rate, concentration and molecular weight as might be expected if the flow elongates the molecules, reduces their entropy and so increases the effective dissolution temperature. It should be possible to see crystallisation under flow temperatures where the crystals redissolve when flow stops.

The most remarkable morphological observation is that shish kebabs grown under extensional flow do not look any different from those grown from turbulent stirred solutions. Several models for the formation of these structures can be envisaged. Mackley and Keller seem to favour formation of an extended chain shish nucleus on which folded-chain lamellar kebabs subsequently grow. Alternatively the shish kebab could be a development of the stacks of polymer crystals formed from static solutions, where the stacks resemble either an open book or a spiral staircase. If the gaps between crystals were opened up these would look like shish kebabs. Nagasawa and co-workers (IUPAC Symposium on Macromolecules, Aberdeen, 1973) reported the formation of shish kebabs by deformation of single crystal stacks and suggest that the shish kebab structures produced from flowing solutions are also the product of the mechanical disruption of stacked single crystals by the fluid flow. This idea would explain the similarity of structures produced under different conditions, the amount of connecting material between kebabs and the general irregularities of shish kebabs. This question could be resolved by studies of how shish kebabs grow.

Thus this elegant work will add to our understanding of flow in polymer solutions and thermodynamic aspects of crystallisation but tells us little about the crysallisation process. Methods are still needed to study the molecular arrangement in these structures. Results on crystallisation of polymer melts in the same apparatus should be as interesting and more directly relevant to polymer processing methods such as fibre spinning. In this respect this simple, controllable crystallisation system could be very enlightening.

Halley lecturer produces new theory of comet origins

by John Gribbin

ON May 6 the 1975 Halley Lecture was presented in Oxford by W. H. McCrea, Professor Emeritus of the University of Sussex. McCrea took as the title of his discourse "The Solar System as a Space Probe", a far-ranging topic which suggests both an explanation of ice ages and a model for the origin of comets.

As McCrea pointed out, the advent of the space age has renewed interest in the study of the Solar System, but in many ways the Solar System itself is the best space probe of all, travelling at present towards a point in the constellation Hercules. The speed of the Solar System — 20 km s^{-1} relative to the rest frame of local stars; 140 km s^{-1} relative to the so-called 'high velocity' stars — may seem small compared with the speeds that are necessary to cross the Galaxy in a reasonable time, by human standards. But the great age of the Solar System, some 5 x 10^{9} years, suggests that even at these modest speeds the system has already orbited around the Galaxy 20 times. So there has been ample scope for samples to have been gathered from different regions of the Galaxy.

The most obvious features of spiral galaxies, like our own, are the spiral arms (made up of bright stars) and the associated dark lanes of gas and dust. Can the passages of the Solar System through these features have left traces for us to discover? McCrea suggests that they have. The spiral arms are thought to be a more or less permanent feature, with the dark lanes along their edges showing the formation of shock waves as clouds of gas run into the arms.

In our Galaxy, there are two main arms, and in round terms the time scales associated with the motion of the Solar System produce a passage across one arm every 100 Myr. It takes 10 Myr to cross each arm, and 1 Myr to cross the lane of shocked and compressed clouds at the edge of the arm. Now, the Sun is just entering the Orion arm, having recently passed through the compression lane which lies just inside that arm.

Following in illustrious footsteps (including those of H. Shapley in 1920 and 1949, F. Hoyle and R. Lyttleton in 1939) McCrea suggests that it is just such passages of the Solar System through the dark lanes which bring about ice ages. Details of this new variation on an old theme are shortly to be published in *Nature* (in our issue marking the 300th anniversary of the foundation of the Royal Greenwich Observatory).

But the astronomers among McCrea's audience really began to sit up and take notice when he moved on to discuss the problem of the existence of comets. These enigmatic objects, with masses in the range $10^{16}-10^{18}$ g, are the least massive members of the Solar System (except for small asteroids) but in many ways the most baffling. Have they always formed part of the Sun's family, or are they ephemeral visitors snatched from the depths of space? Most theorists seem to lean towards the former view; but McCrea has now come up with a model which might change that consensus.

The most popular concept of a comet (but not the only model) sees it as a 'dirty snowball' of water ice and silicates. As McCrea notes, this composition is much the same as the best available models of interstellar grains, which are now regarded as silicates covered by ice. So, if a ball of interstellar dust could be separated out from the gas, we would have something very much like the nucleus of a comet. The snag is that the presence of the gas will tend to keep the interstellar grains apart even in a situation where with no gas they would congregate under their mutual gravitational attractions.

McCrea gets round this by suggesting that dust can collapse independently of the gas if the mean free path of a gas molecule (relative to dust grain encounters) is longer than the minimum radius of a cloud of dust at the same density which would collapse if free from gas. Putting in the numbers, he comes up with a range of masses for the resulting aggregations, and these are just in the range of comet masses. For example, grains 0.25 μm across will form aggregates with mass about 1.6 x 10^{18}g. But to set the whole process off, the gas–dust mixture must be compressed to densities rather greater than the typical density of matter in intestellar space.

Once again, this is where the interaction of the moving gas with the spiral arms comes into the picture. 'Normal' clouds, says McCrea, have densities of about 0.1 – 10 molecules cm^{-3}. Dense clouds have about $10^{5}-10^{7}$ molecules cm^{-3} over regions one or more parsecs across, and the compression lanes must be at least as dense as the dense clouds. When a normal cloud hits a compression region, the first product is likely to be a dense cloud. At successive further compressions as the cloud continues its journey around the Galaxy and across spiral arms first cometary nuclei, then stellar and planetary condensations and even clusters of main sequence stars could be formed, depending on the starting density of a particular cloud. On that picture, the comets which now form part of the Solar System are a by-product of the recent passage through a compression lane, captured by the Sun en route. It may be that we are lucky to see any comets at all, and that for most of the long history of the Solar System there have been no comets bound to the Sun. Those we have at present will soon be dispersed, or broken up by repeated passage into the inner Solar System and past the Sun.

All this is dramatic enough. But the implications of the quite profound changes on Earth which occur as the Solar System moves through different regions of the Galaxy go still further. These "cosmic seasons", as Shapley dubbed them, could well have influenced the development of life, which (in the form we know it) may depend not just on the distance of a planet from its star, but also on the distance of the star from the galactic centre. McCrea terminated his lecture with the comment that the effect of all these astronomical influences on life "can be very complicated".

review article

Mutation selection and the natural history of cancer

John Cairns*

Survival of the rapidly renewing tissues of long-lived animals like man requires that they be protected against the natural selection of fitter variant cells (that is, the spontaneous appearance of cancer). This article discusses three possible protective mechanisms and shows how they could explain various features of the natural history of certain common cancers of man.

We are accustomed to thinking of the combination of natural variation and natural selection as a force for the good, that creates and maintains the fittest in a species and discards the unfit. This is the fundamental theorem of biology. But when we turn from the competition between the individuals of a species to the competition between the individual cells within a single animal, we see that natural selection has now become a liability. The dangerous mutations are now those that confer on a cell an increased survival advantage. We may therefore expect to find, especially in animals which undergo continual cell multiplication during their adult life, the evolution of mechanisms that protect the animal from being taken over by any "fitter" cells arising spontaneously during its lifetime—that is mechanisms for minimising the rate of production of variant cells and for preventing free competition between cells.

The cells of the surface epithelia that separate a typical mammal from its environment undergo continual turnover, and this turnover accounts for most of the cell division that occurs in the life of the animal. For example, a newborn rat contains some 3×10^9 cells and grows to a final value of about 6×10^{10} cells in old age; excluding any programmed cell death, the rat therefore represents a total of 6×10^{10} cell divisions. During its entire lifetime, however, the rat produces and discards about 10^{13} epithelial cells from its small intestine alone, and almost as many cells from various other epithelia[1]. Its risk of being overwhelmed by variants is, in principle at least, related to this total of 10^{13}. For man, who lives ten times as long and is one hundred times as large, there must be some 10^{16} cell divisions, and this number must somehow be reconciled with a spontaneous mutation rate of about 10^{-6} per gene per cell division[2–4]. Because most of the cell division is occurring in epithelia, that is where we may expect to find the protective mechanisms most highly developed.

Our interest in these protective mechanisms arises not least of all from the fact that they will determine how "fitter" variants are created by mutagenic agents and how such variants can be helped to supplant their normal neighbours (the process of carcinogenesis). Here we come to an added reason for concentrating on epithelia. Although most experimental cancer research, especially that using oncogenic viruses, deals with tumours of supporting tissues and blood-forming cells (the sarcomas and leukaemias), human cancer is predominately a disease of the various surface and glandular epithelia (Table 1).

This paper describes various ways in which the programme of cell renewal in epithelia may have evolved to minimise the risk from somatic mutation, and finally how certain features of natural carcinogenesis could be explained in terms of such a programme.

Lineages and the place of stem cells in programmes of cell renewal

The turnover that occurs in the self-renewing epithelia is the result of continual shedding of superficial cells balanced by continual multiplication of the deeper cells. In the simplest examples like skin, cell division is restricted to the deepest (basal) layer of cells. To keep the number of basal cells constant, one of the two daughter cells resulting from each cell division must on average remain in the basal layer and the other must escape and be discarded. Obviously there are several possible programmes of cell division that could produce this result: a regular programme of asymmetric division, where one of each pair of daughter cells is invariably exported (Fig. 1*a*); or a random choice of cells for export, the rule being simply that the available space for basal cells should be kept fully occupied; or lastly some more complex programme in which cells destined for export are allowed to undergo a certain number of divisions before they are exported from the basal layer (Fig. 1*b*). Some elegant experiments on the fate of pairs of daughter cells, arising in the rat oesophagus, suggested that the choice was random[6]. It now seems more likely, however, at least in skin[7,8] and small intestine[9], that a fairly rigid programme is operating of the kind shown in Fig. 1(*b*)—that is, most of the dividing cells produce descendants that are all ultimately exported.

An arrangement of this kind will decrease the rate of accumulation of spontaneous mutations and the risk from exposure to mutagens. The only mutations that accumulate during the life of an animal are of course those that arise in cells that themselves survive or contribute descendants that survive for the animal's lifetime—that is, the mutations must occur in the "immortal" stem cells (drawn as squares in Fig. 1). Any muta-

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tion that arises in a cell destined to be discarded will be lost (unless of course its effect is expressed quickly and serves to prevent the cell from being discarded).

The smaller the proportion of cells that are immortal stem cells, the lower the risk. In the different self-renewing systems of mammals the proportion probably ranges from one in ten (mouse skin)[8] to one in several thousand (bone marrow)[10]. But in most instances the number of immortal stem cells is not known. Note, however, that it may be much less than the number of cells capable of being promoted to immortality by force of circumstances (for example, that are capable of recolonising an epithelium or bone marrow depleted by X irradiation) or the number of cells that are still undifferentiated (the usual, but in this context less useful, meaning of the term "stem cell").

Stem cell division and the segregation of old and new DNA strands

Although the division of each immortal stem cell can produce two apparently indistinguishable daughter cells the fate of which may, in many systems, be determined simply by their position in space, it does not follow that the two daughters must be genetically equivalent and must therefore be equally likely to contain mutations.

Most spontaneous mutations probably arise as errors during DNA replication. Because DNA is duplicated semi-conservatively, these mutations are present initially in "heterozygote" molecules with one mutant strand and one normal strand. Unless such a heterozygote molecule is converted to homozygosity by one of the DNA repair mechanisms of the cell it will, on subsequent duplication, yield one normal daughter molecule and one molecule which is mutant in both strands (Fig. 2*a*). We see at once that stem cells would be protected against errors of duplication if it were so arranged that the immortal daughter cell always receives the DNA molecules which have the older of the two parental strands (that is, the rightmost molecules in Fig. 2*a*) and the mortal daughter always collects the molecules with the younger parental strand (that is, always collects the mistakes made in the previous generation). This rule would therefore maintain an "immortal strand" through successive generations.

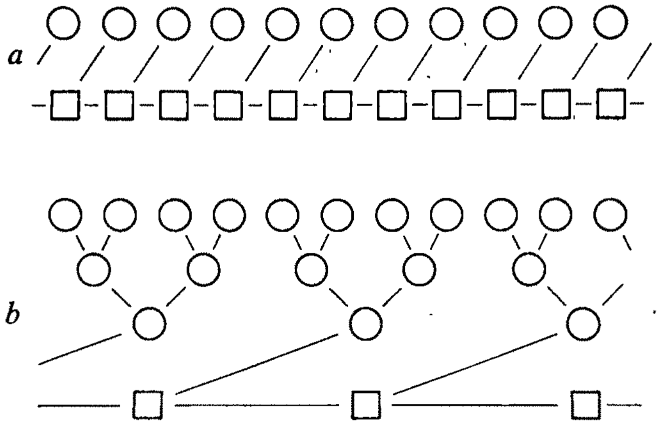

Fig. 1 Two possible programmes for the renewal of an epithelium by multiplication of the cells in the deepest (basal) layer, *a*, Renewal simply by regular, asymmetric division of stem cells; *b*, renewal by asymmetric division of the stem cells, plus some further division of the cells destined to be exported to the surface. The immortal stem cells are shown as squares and the mortal, differentiating cells as circles.

To operate such a rule, the immortal strands must all be marked in some way so that sister chromatids can be distinguished at the centromere, and all centromeres must behave in a coordinated fashion. Also, if the rule is to apply to all parts of the genome and not merely to the centromeric regions, the cell must ensure that sister chromatid exchanges are rare.

Table 1 Cancer incidence in Denmark, 1943–67

Type	Commonest sites	Total cases	%
Carcinomas			
External epithelia	Skin, large intestine, lung, stomach, cervix	168,591	56
Internal epithelia	Breast, prostate, ovary, bladder, pancreas	110,182	36
Sarcomas and leukaemias		23,801	8
	TOTAL*	302,574	100

These figures are drawn from three reports that together summarise the experience of the 4.5 million inhabitants of Denmark over a period of 25 years (ref. 5). They were chosen here because, unlike most national registries, the Danish Cancer Registry reports separately for each site the number of carcinomas arising in the epithelial (parenchymatous) cells and the number of sarcomas arising in the supporting cells.

* This total excludes about 10,000 cases in which the exact site of the primary cancer was not known.

In addition, if the fate of the two daughters of any immortal stem cell is determined by their position in space, the rule requires that each stem cell must enter mitosis with its duplicated chromosomes correctly aligned with respect to its surroundings. Up to a point this is certainly true. For example, in skin the plane of division of the basal cells is so arranged that both daughter cells initially lie side-by-side in the basal layer; further, there is some evidence that the successive mortal daughters of each stem cell lie alternately on one side and then on the other side of an unchanging plane of division[8]. In fact, such a pattern would fit nicely with the observation that when a given chromosome is followed through repeated rounds of duplication, in a cell forced to undergo endoreduplication by being grown in colchicine, each parental strand is found to have segregated first to one side of the plane of division and then to the other (Fig. 2*b*)[11].

Unlike the matter of stem cell lineages which is reasonably well understood, this speculation about the relationship between stem cell lineage and DNA lineage is completely unsubstantiated. In many instances, cells labelled in their DNA during one cycle have been shown to divide this label unequally among their grand-daughters[12], and there is one example of a cell with several chromosomes (the germinating spore of *Aspergillus*) which regularly segregates the DNA strands in these chromosomes as if they are parts of a single DNA duplex[13]. It remains to be shown, however, that epithelial stem cells behave in this way. The test, of course, is to see whether stem cells can be permanently labelled in their DNA at the time they are first formed (that is, can be shown to have a DNA strand that is immortal) or alternatively to see whether they retain the label only for one generation when labelled in an adult animal (that is, normally discard the newer of their parental DNA strands). Experiments of this kind are not yet completed.

Compartmentalisation and the natural selection of fitter variants

Having discussed two arrangements that would reduce the effective mutation rate in multicellular animals, we can now turn to a factor influencing the selective forces operating on mutant cells.

Most experimental cancers are formed only after repeated or prolonged exposure to a carcinogen. Similarly, most human cancers appear in old age, as if they usually arise as the end result of a lifetime of carcinogenesis. For this reason it is generally believed that several steps are required to convert a normal cell into an uncontrolled, invasive variant. The exact number of steps could be determined from the relationship between age and incidence provided that all steps occur at the same, unchanging rate[14,15]. If, however, the first step increases the survival of the altered cell so that this cell type steadily increases its number at the expense of normal cells, there will be a corresponding steady increase in the chance that somewhere there is a cell undergoing the next step, simply because with time there will be more and more first-step cells at risk[16,17]. So, whatever the number of restraints that must be overcome to convert a normal cell into a cancer cell, the risk that this occurs will be much increased if there are intermediate states with high survival advantage and if these variants are in fact able to displace their neighbours. We may therefore expect to find fast-multiplying tissues arranged in such a way that neighbouring stem cells (or sets of stem cells) are restricted to limited territories so that they cannot easily compete with each other.

The epithelium of the small intestine, which is the site of the most rapid cell turnover in the body, is an example of just such an arrangement. Cell multiplication is confined to indented regions of epithelium (the crypts) that project deep into the underlying mesodermal tissues. Between each neighbouring pair of crypts the epithelium forms a fold (the villus) that projects into the lumen of the gut. As a result of the continuous cell division in the crypts there is a continuous flow of cells from each crypt to the tips of the neighbouring villi, and from there the cells are shed into the gut[18]. Apparently each cohort of cells moves *en bloc* up the villus, propelled by the pressure of new cells being formed in the crypt. No matter how many immortal stem cells are normally present in each crypt (and the number is not known), any variant stem cell with high survival advantage should quickly exclude normal cells from its crypt. Unless this variant is, however, able to alter the whole structure of the epithelium (for example, by invading the surrounding mesoderm) the only way it can reach neighbouring crypts is by moving down the other sides of the villi, against the continual flow of normal cells arising in these crypts.

We see therefore that the arrangement of the intestinal epithelium into crypts and villi will tend to limit the expansion of any population of fitter variants that might otherwise form an ever-increasing target for further mutational steps. Other epithelia are no doubt partitioned in other ways. In each case the rules governing the territoriality of the proliferating cells will determine which kind of variants are the most successful.

Mutation selection and carcinogenesis

Restricting the number of stem cells, imposing a special pattern of segregation of DNA strands, and restraining the opportunities for competition between cells, are just three of the possible ways that may have been evolved in long-lived multicellular animals for diminishing the rate at which mutant cells accumulate. Of course, the rate would be further decreased if stem cells have unusually error-free forms of DNA synthesis and repair, perhaps in conjunction with a prolonged interval between successive divisions to allow ample time for any necessary repairs. The object of this article, however, is not to produce a list of all the factors that might affect mutation rate, but to show how the organisation of the self-renewing tissues of multicellular animals may often determine the sequence of events during carcinogenesis.

We have seen that the lineage of epithelial cells is so arranged that rapid renewal is normally achieved by rather few divisions of a special minority population, the immortal stem cells, which have a somewhat protected position deep in the epithelium and may, in addition, retain specially preserved DNA strands. The rate of accumulation of mutations in such a system will be proportional to the rate of division of the stem cells or perhaps, more specifically, to the rate at which they have to multiply to replace lost stem cells (that is, the rate at which new immortal strands have to be created). Anything that accelerates either of these processes could be carcinogenic. For example, cigarette smoking causes a thickening of the bronchial epithelium and obviously disrupts the ordered programme of cell renewal[19,20]. Judging from the relationship between incidence and age and duration of smoking[21], it seems that the smoker accumulates in each year about the same risk that 50 non-smokers accumulate collectively; alternatively, since incidence increases as the fourth power of time, it could be said that the smoker's bronchial epithelium goes through time at about 2.7 times the normal rate. It is tempting therefore to ascribe the increased risk of lung cancer simply to a 2.7-fold increase either in the rate of division of stem cells in the bronchial epithelium or in the rate at which

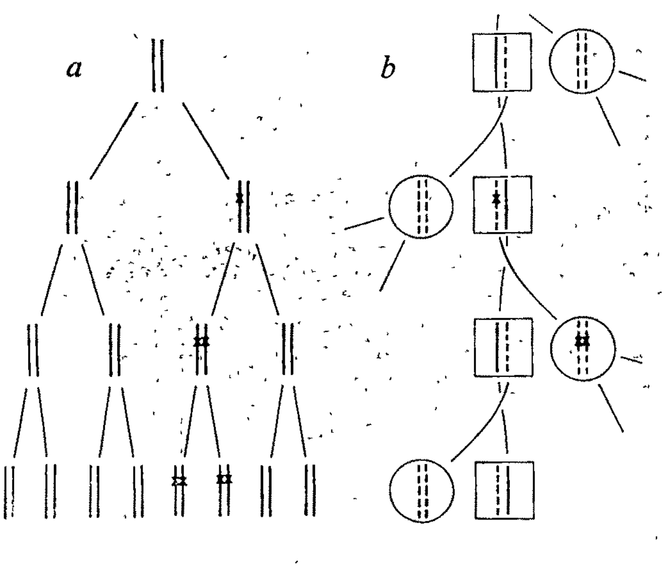

Fig. 2 The lineage of DNA undergoing semi-conservative replication. *a*, The segregation of a mutation, X, that arose as a copying error during duplication. *b*, Rearrangement of this lineage to fit the observation that parental strands apparently tend to segregate alternately to the right and left of the plan of division[11] and that, in one instance at least, the stem cells also move at division alternately to one side and then to the other[8]. The hypothesis therefore is that stem cells (☐) keep the same parental DNA strands through successive cycles.

these stem cells have to be replaced. This example shows that although it is customary to think of carcinogens as necessarily acting directly as mutagens, some could act indirectly simply by altering the programme of division of immortal stem cells.

Nevertheless there is no escaping the fact that the most powerful experimental carcinogens are intense mutagens. So we should now consider what general classes of mutation are likely to lead to cancer. In particular, because most forms of human cancer seem from their age-incidence to be the end-result of several consecutive, independent steps (mutations) we should be especially interested in those mutations that are likely to affect the subsequent rate of formation and selection of additional mutations. The previous example showed how a relatively slight change in the rate of stem cell division may have a great effect on cancer incidence. Obviously the same effect could be produced by an increase in the mutation rate per cells division, resulting from change in a gene with a product concerned with the duplication or repair of DNA. Unless such mutagenic mutations confer some survival advantage, however, they will remain confined to the stem cells in which they arise, and so only if they produce enormously increased mutation rate will they raise significantly the overall mutation rate; for a single cell to double the probability that an organ with 10^8 stem cells has experienced an event which increases as the fourth power of time, that cell would have to move through time (that is, collect mutations) at 100 times the normal rate. Probably more important, therefore, are mutations that affect the interactions of a cell with its neighbours. Any mutation that gives a stem cell the ability to move out of its compartment in an epithelium may cause it to form an expanding clone of stem cells, and because such a clone would be the site of an unusually rapid rate of formation of immortal strands it should show a much higher mutation rate than the rest of the epithelium; the first mutation would therefore be acting as a 'primer' for whatever succession of further alterations are needed to make a fully invasive cancer. The most carefully studied precursor states for any human cancer are the various precancerous abnormalities of the cervix, and they show exactly the properties we would expect of primer mutations. They are characterised by the appearance of extensive clones of cells[22,23] with deranged programmes of cell renewal[24,25]; further, although for obvious reasons the undisturbed natural history of these states is not well quantitated, it does seem that the successive levels of abnormality succeed one another with a rather high probability[26,27], as if the first level of abnormality has indeed raised the rate at which mutant cells accumulate.

If stem cells do preserve an immortal DNA strand through successive divisions, the only time when it should be easy to create a primer mutation (that is, immortalise an error) may be when these immortal strands are being synthesised—that is, when the epithelium is being created or is having to regenerate. It is therefore significant that carcinoma of the cervix usually arises immediately next to the boundary with the cervical canal, where the stratified epithelium of the cervix changes to the columnar epithelium of the canal[28]. The exact position of this boundary is known to fluctuate, and it is easy to imagine that the epithelium here is especially at risk because the local population of each kind of stem cell (stratified and columnar) has to rise and fall with each fluctuation.

Most epithelia probably establish their stem cells *in utero*[29], and so, if undisturbed, are unlikely to form primer mutations. One obvious exception, however, is mammary epithelium which proliferates after puberty with each ovarian cycle[30,31] and further proliferates during each successive pregnancy. In this special case a most interesting relationship is found between the extent of the proliferation that occurs during sexual maturity and the probability that cancer develops in old age[32]. The overall (lifetime) risk of cancer developing in childbearing women seems to be linearly related to the length of the interval that elapsed between puberty and the first full-term pregnancy, rising in value from 30% to about 130% of the value for women who never have children. So there are two unusual features to be accounted for: although the mammary epithelium proliferates at each successive pregnancy, it is only the first pregnancy that affects the risk of breast cancer; second, although the incidence of most cancers (this one included) rises as about the fourth or fifth power of age, the total lifetime risk of getting this cancer proves to be directly proportional to the length of one particular time interval. The explanation could be as follows. First, we suppose that the stem cells of breast epithelium increase in number at the time of puberty and then undergo a certain fluctuation in number with each ovarian cycle until the first pregnancy, whereupon the population builds up to a final level that is no longer influenced by the ovarian cycle or by additional pregnancies. Second, we suppose that the chance of a primer mutation occurring is simply proportional to the total number of times that immortal strands have had to be made in this epithelium (that is, the number of stem cell generations that have occurred)—much in the way that the frequency of mutants in a bacterial chemostat increases linearly with time[33]. Once pregnancy establishes the final unchanging population of stem cells, the epithelium becomes as protected against mutation as any other proliferating epithelium.

For obvious reasons, experimental carcinogenesis is usually studied in systems where brief exposure to a carcinogen produces a high incidence of cancer within a few weeks or months. It could be argued that such systems, using very strong carcinogens, are not suitable models for human cancer which typically arises at a slow rate after an incubation period of many years. Therefore this article, being on the natural history of cancer, has used examples of the common human cancers. The idea that special primer mutations are the first step in the formation of most cancers comes in large part, however, from certain reports of experimental carcinogenesis where the process could be divided into the separate stages of 'initiation' and 'promotion'[34,35].

I thank Drs Robert Kay, Guido Pontecorvo, Christopher Potten and many others for stimulating discussions and criticism.

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article

Recent African rainfall patterns

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We present the results of a pilot study aimed at creating a more concrete basis for discussion of the variability in both space and time of rainfall in the Sahel.

FOR the purpose of this report we define the Sahel region as that running across the entire African continent between 10°N and 20°N. The area west of approximately 30°E is a fairly regular plain; to the east are the Nile River basin of Sudan and the mountains of Ethiopia.

The atmospheric circulation system that brings rain to this region is the ascending branch of the tropical Hadley cell, often called the Intertropical Convergence Zone (ITCZ), which moves north of the equator in the Northern Hemisphere summer. This system usually reaches its most northerly position in August so that this is the wettest month over the entire broad region of the Sahel[1]. We have concentrated on August for the African region for the years of good climatic records, 1941–73.

The data for this study were derived from the published monthly records[2] and a magnetic tape of global surface data before 1971 furnished by the National Center for Atmospheric Research. Meteorological agencies of the United Republic of Cameroon, Ethiopia, the Republic of Ghana and the Republic of Mali provided supplementary data.

Figure 1 illustrates the average rainfall patterns for August for the African region for the periods 1941–50 and 1951–60. The stations which usually report are indicated by dots. A similar map for the 10-year period 1961–70 has not been produced at this time because of important gaps in reporting. Several features of these maps are important. The Sahel region had about 10% more rainfall in 1951–60 than in the earlier decade; the centre of the maximum precipitation was approximately 1° farther south in the earlier decade than in the latter; the August rainfall belt across approximately 15°N has two distinct regions, one covering the large plains in western and central Africa and the other in the proximity of the Nile basin and Ethiopian highlands; and there are two areas of orographic rainfall suggested by the small areas averaging more than 600 mm of precipitation for the month.

In Fig. 2 we show analyses of the August precipitation data for 1964, 1968 and 1972. That for 1964 shows a month of relatively heavy rainfall over the Sahel. The pattern is quite similar to those of 1951–60; 1968, on the other hand, is the year in which the belt of maximum precipitation underwent the smallest northward displacement in the 33 yr of adequate record, to only about 7.5°N, compared with the average of about 12.5°N for 1951–60. Nevertheless, August 1968 was not the driest recorded in the Sahel, which suggests that the meridional gradient of precipitation was flatter in this month than in other drier months. Another indication of the uniqueness of this month is the region of heavy, flooding rainfall along the normally dry southern coast between 10°W and 0° longitude[3].

The years 1972 and 1973 were those of lowest precipitation in 1941–73 in the Sahel. This is clearly indicated by the break in zonality of the 200 mm isohyets along 15°N in the map of August 1972.

Figure 3 illustrates a relatively crude attempt to show meridional 'cross sections' of the rainfall pattern across the Sahel, derived from a series of eight stations along approximately 0° longitude, for August of 1964, 1968 and 1972 and the long term mean. The figure suggests that August 1964 was indeed wetter than the mean in the Sahel region but drier in the region between 5°N and 10°N. The curve for August 1968 further illustrates the relatively limited northward migration of the zone of maximum precipitation inferred from Fig. 2. August 1972 seems to have had decreased rainfall for the whole region illustrated, with a centre of maximum precipitation slightly south of that of the mean.

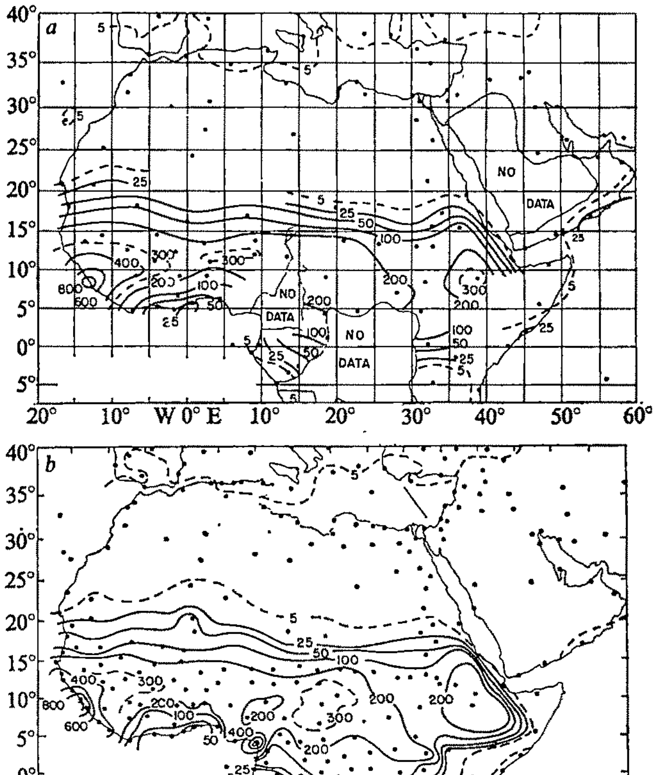
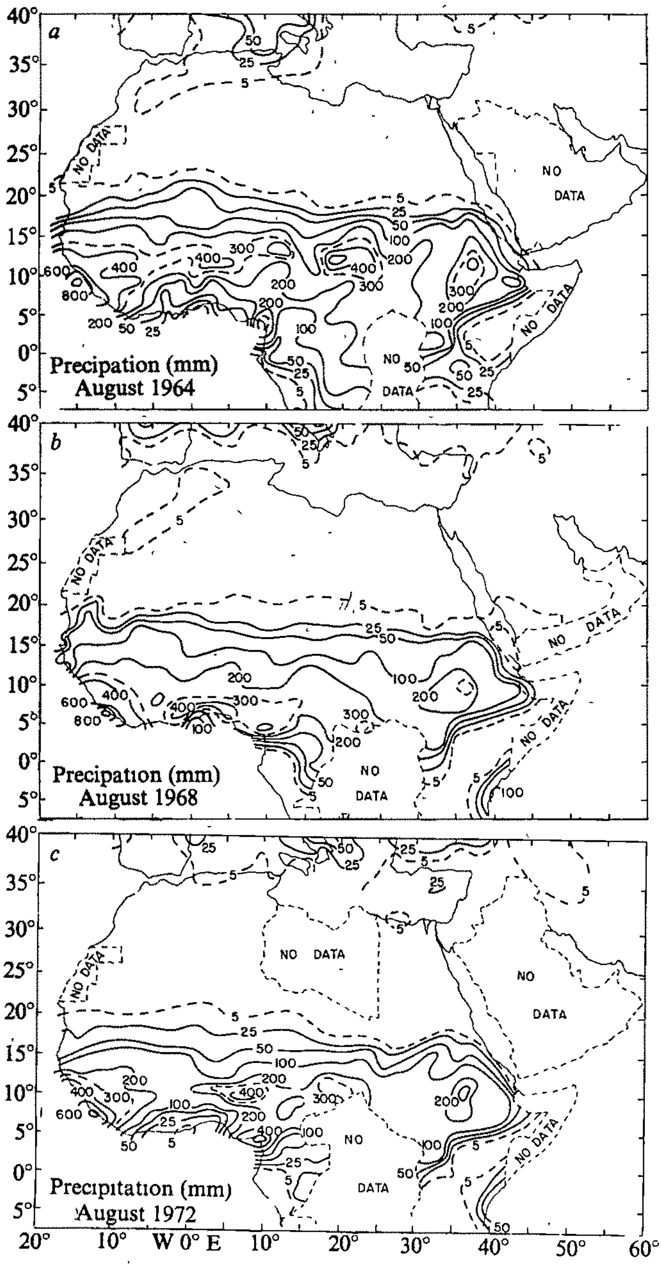


Fig. 1 Analyses of average August precipitation data (mm) for 1941–50 (*a*) and 1951–60 (*b*) for the African region. Dots locate positions of stations which usually report in the respective decade.

Figure 4 portrays five possible measures of the 'intensity of drought' for the Sahel. The lower two curves show the arithmetic mean August and annual precipitation of eight stations along 14°N. The stations were picked because of their proximity to the chosen latitude, their relatively equal amounts of August and annual rainfall, and their nearly continuous records since 1941. El Fasher was chosen as the most easterly station considered because data from sites near the Nile basin and Ethiopian mountains tend to obscure the clear trends in the region west of about 30 °E (Figs 1 and 2). These curves show that the patterns in time of August and annual data are very similar. The wettest five-year period was that from 1950 to 1954, which had about 120% of the long term annual mean. The driest was 1968–72, with about 80% of that mean.

The middle curve of Fig. 4 shows a line drawing of the average August precipitation along 15°N between 10°W and 25°E obtained from the entire set of analysed maps. The curve directly above indicates the average latitude of the 100 mm isohyet in the Sahel between 10°W and 25°E as read from the same maps. The lines for the 1951–60 means are the result of similar readings of the 1951–60 map (Fig. 1). The top curve of Fig. 4 indicates the approximate latitude of the centre of maximum rainfall (centre of gravity) near the prime meridian as interpolated from the August analysed maps. Figure 4 clearly shows that 1968–73 was the driest six-year spell of the period covered by good records.

Fig. 2 Analyses of August precipitation data for 1964 (*a*), 1968 (*b*), and 1972 (*c*) for the African region.



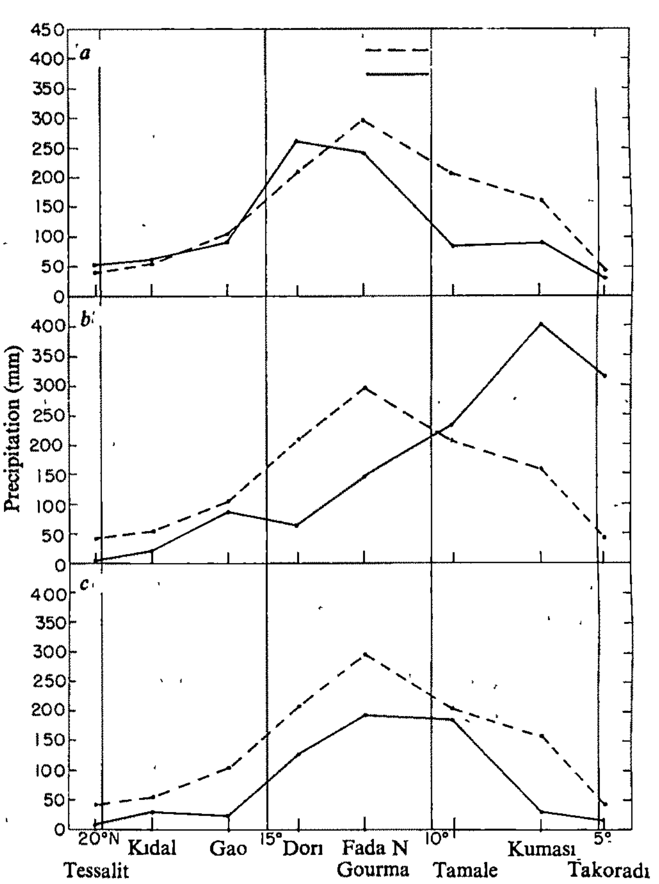


Fig. 3 Plots of August precipitation data for the eight stations listed, which are approximately along the prime meridian, for 1964 (*a*), 1968 (*b*), and 1972 (*c*), together with the long term mean. Station positions are: Tessalit, 20°12′N, 0°59′E; Kidal, 18°26′N, 1°32′E; Gao, 16°16′N, 0°37′W; Dori, 14°2′N, 0°3′E; Fada N Gourma, 12°04′N, 0°21′E; Tamale, 9°25′N, 0°53′W; Kumasi, 6°43′N, 1°37′W; Takoradi, 4°53′N, 1°46′W. — — — —, Climatology; —, precipitation.

The slight discrepancies between the five curves further indicate the difficulty in determining a single quantitative measure of regional precipitation patterns.

The 12-month running means of the entire record of several stations having data for the years before 1941 (including Accra, 5°33′N, 0°12′W; El Fasher, 13°38′N, 25°20′E; Kano, 12°3′N, 8°32′E; Khartoum, 15°36′N, 32°33′E; Lungi, 8°37′N, 13°12′W) were computed. Taken as a whole, these tend to confirm Jenkinson's[4] conclusion that there was a severe drought for a three-year period centred on 1913 and a less severe one around 1926. In addition September volume flow measurements[5] of the Niger River at Kayes indicate that the flow in 1913 was about 28% of the mean for 1903–68, the lowest reported up to 1968. The flow in 1926 was 59% of the mean. The running means were also compared to the curves of Fig. 4. Unfortunately, each station failed badly to correlate with the curves in at least one distinct year with the exception of Kano, which has an unfortunate lack of data in the critical period after 1966. This finding, combined with the fact that there are only a few poorly distributed stations in the Sahel for the earlier period, seems to preclude a comparison of the severity of earlier droughts with the most recent for the broad Sahelian region.

Spatially, both the position of maximum rainfall and the northward gradient from that position are necessary to specify the precipitation pattern over the Sahel. In addition, the

Table 1 Climatic data from analysed global maps

	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Global average 700-mbar height at 50°N in January	2,850	2,879	2,879	2,864	2,857	2,838	2,859	2,870	2,870	2,840	2,867	2,837	2,872
Global average 700-mbar height at 50°N in August	3,064	3,053	3,061	3,041	3,044	3,057	3,054	3,045	3,053	3,058	3,052	3,065	3,069
700-mbar height over the Sahara at 25°N in August	3,215	3,220	3,205	3,215	3,208	3,220	3,215	3,240	3,240	3,230	3,220	3,215	3,230
700-mbar height over the Atlantic at 25°N in August	3,209	3,200	3,210	3,210	3,215	3,210	3,220	3,210	3,210	3,215	3,210	3,220	3,210
Latitude of 700-mbar high over the Sahara in August	30°N	32°N	30°N	29°N	30°N	27°N	30°N	27°N	25°N	30°N	30°N	25°N	30°N
Latitude of 700-mbar high over the Atlantic in August	32°N	33°N	33°N	33°N	30°N	32°N	32°N	27°N	32°N	32°N	33°N	32°N	32°N

patterns of precipitation for the large area of plain west of about 25°E are only related to those east of that longitude in a complex fashion. This is perhaps not surprising if one considers the orography of the eastern region and the difference in average wind patterns of that region compared to those of the central and western Sahel[1]. So Khartoum and other Sudan stations are not representative of the large scale trends and are therefore probably of little value in estimating trends before 1941. Finally, the 1968–73 period was indeed the most distinctive since 1941, although the period around 1913 may be comparable. Preliminary reports suggest that Sahelian rainfall was near normal in 1974. The eight-station average for August 1974 is indicated in Fig. 4.

Winstanley[6] has suggested that Sahelian rainfall is negatively correlated with the southernmost position of the troughs in the mid-tropospheric circumpolar westerlies as inferred from winter-season 500-mbar analyses at 40°N between 110°W and 70°E. His results have been interpreted as a migration of the Sahara southwards during 1968–73. We have prepared 700-mbar height analyses for January and August for the years 1961–73. From these, values were read at each 10° meridian around 50 °N. The averages of these heights are given in Table 1. Given the relative randomness of the results for January and the large standard deviations within a latitude circle (about 95 geopotential metres, gpm) there does not seem to be any trend in these heights which could be correlated with Sahel rainfall trends (see also ref. 7).

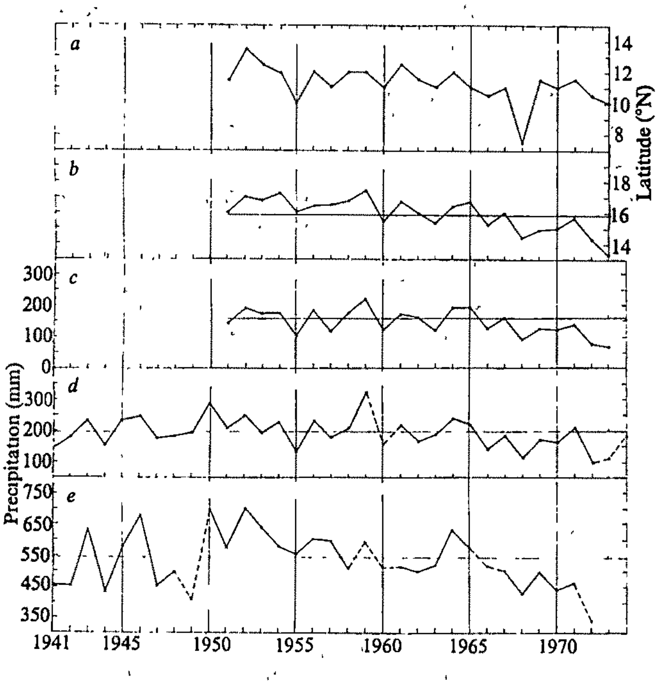


Fig. 4 Plots of five measures of rainfall for the Sahel region from 1941 or 1951 to 1973. The data of the upper three curves were read from analysed August precipitation maps. The bottom two curves indicate the averages of the station reports at: Tambaconda, 13°46′N, 13°41′W; Kayes, 14°26′N, 11°26′W; Mopti Severe, 14°31′N, 4°6′W; Gao, 16°16′N, 0°37′E; Niamy, 13°39′N, 2°10′E; Zinder, 13°48′N, 9°0′E; Abecher, 13°51′N, 20°51′E; El Fasher, 13°38′N, 25°20′E. Dashed lines indicate one station report has been objectively interpolated using the long term means and that year's actual reportings for the other seven stations. *a*, Latitude of centre of gravity, 5°W–5°E; *b*, August latitude of 100 mm precipitation, 10°W–25°E (solid line, 1951–60 mean); *c*, August precipitation along 15°N, 10°W–25°E (solid line, 1951–60 mean); *d*, eight-station mean August precipitation (solid line, 1941–70 mean); *e*, eight-station mean annual precipitation (solid line, 1941–70 mean).

In Table 1 we also present estimates of the latitude of the August subtropical high and the 700-mbar height at 25 °N in the regions of the Sahara and the central Atlantic. Neither the Atlantic data nor the August global averages of 700 mbar height at 50 °N (standard deviations about 30 gpm) show a clear trend. The Atlantic estimates would not support Bryson's[8] contention that Sahelian rainfall patterns can be inferred from the position of the subtropical high over the Atlantic. Namias[9], on the other hand, has reported evidence of negative correlations between the geopotential over northeast Europe and the rainfall for three stations in the Sahel for the summer season. Both the latitude of the subtropical high and its height at 25° N north of the Sahel suggest a southerly migration of that high by about 2° of latitude from 1961–65 to 1969–73. The cumulative regional and global geopotential evidence suggests a slight change in the morphology of the subtropical high pressure belt and the trough of the mid-latitude westerlies during 1968–73, but does not seem to be consistent with a suggestion of a global expansion southward of either.

Available German Weather Service global surface temperature departure maps[10] have been supplemented by our own plots of the station data to provide analyses for January, July and August of 1961–73. The August temperatures of the Sahel were warmer during 1968–73 than the long term mean, with departures exceeding 2 °C in 1968 and 1972. Satellite measurements suggest that this can be attributed, in part, to increased solar heating at the surface due to the decrease in cloudiness and consequent change in overall albedo during this relatively dry epoch. In 1969, for example, the total albedo over the Sahel was about 35% in July and 26% in the following dry month of October[11].

We thank the United States National Science Foundation for support.

Received February 6; accepted March 25, 1975.

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letters to nature

Magnetic field of Mercury confirmed

On March 16, 1975, the US spacecraft Mariner 10 underwent its third and final encounter with Mercury. This unique and fortuitous event occurred because the heliocentric orbital period of Mariner 10 was 176 d, exactly twice that of the orbital period of Mercury. The principal objective of the third fly-by was to confirm or reject the suggestion from the first fly-by on March 29, 1974 that Mercury may possess a modest intrinsic magnetic field sufficient to deflect the solar wind flow[1-2]. The quantitative analysis[3] of the Mercury I data yielded an estimate of the planetary magnetic dipole moment equal to 5.1×10^{22} gauss cm^3 and oriented 7° from the orbit normal. The spacecraft had not been functioning properly since Mercury I because of various technical failures, but the spacecraft engineering team at the NASA Jet Propulsion Laboratory (JPL) managed to achieve the desired objective at Mercury III of a very close dark-side pass. Here we present preliminary results from the NASA Goddard Space Flight Center magnetometer instrumentation on Mariner 10.

The fly-by trajectory is shown in planet centred solar ecliptic coordinates in Fig. 1. This is a preliminary trajectory and will eventually be updated by means of tracking observations made after the encounter. The nominal 'miss distance' at closest approach was approximately 323 ± 8 km. For comparison, also shown in Fig. 1 is the trajectory at Mercury I encounter, for which the miss distance at closest approach was 704 km. In Fig. 1*b*, showing the view from the Sun, the much higher latitude of the spacecraft pass during Mercury III is well illustrated. This closer and more poleward targeting was chosen so as to yield magnetic field measurements which would provide unequivocal evidence about the nature of Mercury's magnetic field. The Mercury I results were consistent with a reduced-scale model of the interaction of the solar wind with the terrestrial magnetic field, in which the planetary field is compressed by the solar-wind flow (itself deflected around the planet) and a detached bow shock wave forms. As a result, the field is then confined to a region close to the planet on the Sunward side and extended to form a magnetic tail on the night side.

Based on the observations and analysis at Mercury I and assuming similar conditions of solar-wind momentum flux at Mercury III, predictions were made of the locations of the characteristic bow shock and magnetopause boundaries (Fig. 1*a*). Since the rotation period of the planet is uniquely coupled to its orbital period, in the ratio 2:3, the 'phase' of the planet relative to the planet–Sun line at successive encounters was identical.

The observed positions of the bow shock and magnetopause during Mercury III are also illustrated in Fig. 1. They show excellent agreement with the model boundaries, which used a scale magnetic moment of Mercury equal to 7×10^{-4} that of the Earth's dipole moment. In the case of the solar-wind flux impinging normal to a dipole field, a theoretical study[4] has yielded the relationship

$$R_{mp}/R_p = 1.07\,[B_0^2/4\pi mnV^2]^{1/6}$$

where R_{mp}/R_p is the normalised radius of the stagnation point distance of the magnetopause, B_0 the equatorial dipole field intensity, and mnV^2 the solar-wind momentum flux (where n is the number density, m the mass of the proton and V the velocity of the solar wind). The distance to the magnetopause, as measured by R_{mp}, thus depends weakly on the solar-wind momentum flux and, therefore even if variations of ±60% in the solar-wind flux occur, they would alter the position of the characteristic bow shock and magnetopause boundaries by only ±10%.

The magnetic field measurements were accomplished with a dual magnetometer system[5] which sampled the vector magnetic field 25 times a second, with a precision of $\pm 0.125\,\gamma$ for each orthogonal component. These data are to be considered preliminary in that they are accurate to ± 1–2γ or ±1%, whichever is the larger, because they are derived from quick-look data provided in near real time by the JPL. Six-second averages of the observed magnetic field throughout the encounter period are

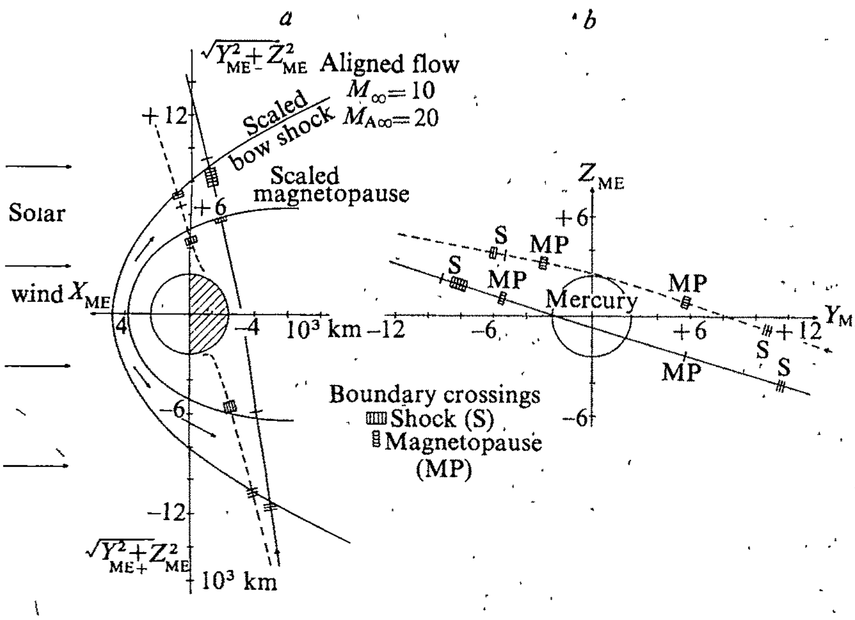

Fig. 1 Mariner 10 spacecraft trajectory in Mercury-centred solar ecliptic coordinates. *a*, Trajectory plotted as distance, X_{ME}, from the dawn–dusk terminator of the planet against distance from planet–Sun line, $(Y_{ME}^2+Z_{ME}^2)^{1/12}$. View from the Sun with Z_{ME} positive to north ecliptic pole. – – –, Mercury III; ———, Mercury I.

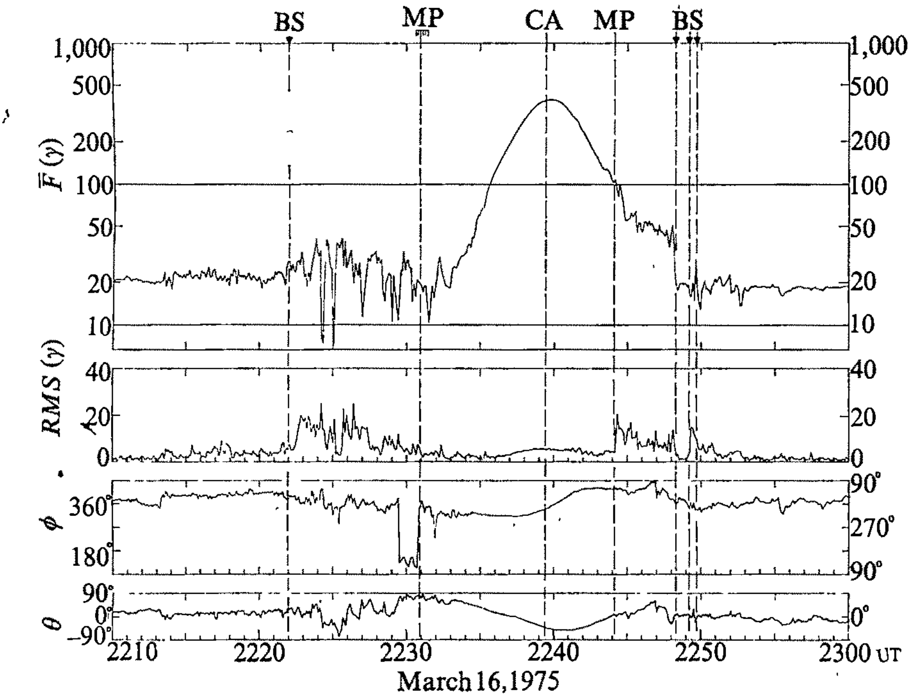

Fig. 2 Measurements by the NASA GSFC magnetometer on Mariner 10 during Mercury III. Magnetic field direction latitude, θ, is referenced with respect to a plane parallel to the ecliptic and longitude, φ, with respect to the planet–Sun line reckoned positive relative towards the east limb of the Sun. BS, Bow shock; MP, magnetopause; CA, closest approach.

shown in Fig. 2. The occurrence of the well developed bow shock and magnetopause boundaries are indicated and readily identified by a magnitude and/or directional change as well as in the Pythagorean mean fluctuation characteristic, *RMS*. The maximum magnitude of the field is 400 γ, four times larger than that observed at Mercury I encounter and 20 times larger than the interplanetary field, which is close to 20 γ. This large value precludes any reasonable possibility that the magnetic barrier to solar-wind flow is associated with a complex induction process occurring at the planet. The direction of the magnetic field, as measured by latitude θ and longitude φ, is primarily towards the Sun (and planet), with gradual changes along the trajectory within the magnetosphere boundaries. This orientation is opposite to that observed at Mercury I but is consistent with the model of a Mercurian magnetosphere (Y.C.W. and N.F.N., unpublished) in which the planetary field is represented by a centred dipole normal to the flow of the solar wind and which is then highly distorted by magnetopause currents.

Table 1 Comparison of planetary dipole moments obtained by spherical harmonic analysis of magnetic field data

	Mercury I March 29, 1975	Mercury III March 16, 1975
Dipole moment (gauss cm^3)	5.1×10^{22} ($350\gamma\ R_m{}^3$)	$4.8 (\pm 0.5) \times 10^{22}$ ($330 \pm 30\gamma\ R_m{}^3$)
Latitude (ME coordinates)	−80°	−76° ± 5°
Longitude (ME coordinates)	285°	90 ± 30°
Root mean square residual	0.9 γ	7 γ
Comment	Quiet data only	Preliminary but entire data set

A preliminary spherical harmonic analysis of the data within the magnetosphere has been carried out using the available trajectory. The analysis assumes a centred planetary dipole field (harmonic order $N = 1$) as well as external field sources described by harmonic terms up to order $N = 2$. The preliminary least-squares fit yields a residual of 7γ with the deduced parameters for the planetary dipole listed in Table 1.

These are compared with those values derived from Mercury I and are seen to be in remarkably good agreement, considering the preliminary nature of these results. The magnitude of the dipole moment is the same within 10% and its direction only 24° from that derived earlier. The uncertainty associated with the present analysis means that we cannot attribute any significance to these small differences.

In summary, the observations at the third encounter with Mercury by the NASA GSFC magnetic field experiment have confirmed the earlier tentative conclusion that Mercury has a modest, intrinsic magnetic field, the origin of which is at present uncertain. It may be associated with remanent magnetisation of parts of the planet (material at present below its Curie point) or it may be caused by an active dynamo in the presumably large iron–nickel core. It is, however, difficult to construct a plausible sequence of events leading to the model in which the Mercurian magnetic field is explained in terms of remanent magnetisation, so we conclude tentatively that these observations require the existence of an active dynamo mechanism.

We thank Dr James Dunne, JPL Project Scientist on Mariner 10, and the JPL spacecraft team for their outstanding effort in achieving the third encounter. We also appreciate the special efforts of D. H. Howell, F. W. Ottens and R. F. Thompson of the NASA GSFC for their special efforts and prompt analysis of these data.

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Preliminary interpretation of plasma electron observations at the third encounter of Mariner 10 with Mercury

OBSERVATIONS of electrons with energies between 13.4 and 690 eV made at the first encounter of Mariner 10 with Mercury (Mercury I) unexpectedly showed[1] the presence of a strong bow shock wave, enclosing a magnetosheath and magnetosphere with boundaries the positions of which could be explained by assuming Mercury to possess an appreciable magnetic moment, in agreement with, and complementary to, the observations made by the magnetometer carried by the spacecraft[2]. Inside these boundaries were found distinct distributions of magnetospheric particles.

A second encounter took place on September 21, 1974, but the spacecraft passed the planet on the Sunward side at too great a distance to permit observations inside the planet's bow shock. The third, and last, encounter (Mercury III) took place on March 16, 1975 at which time the spacecraft passed within 330 km of the surface of the planet, along the trajectory shown in Fig. 1. The primary purpose of this close polar approach was to determine whether the magnetic moment of the planet was intrinsic and to enable further observations to be made inside the magnetosheath and magnetosphere.

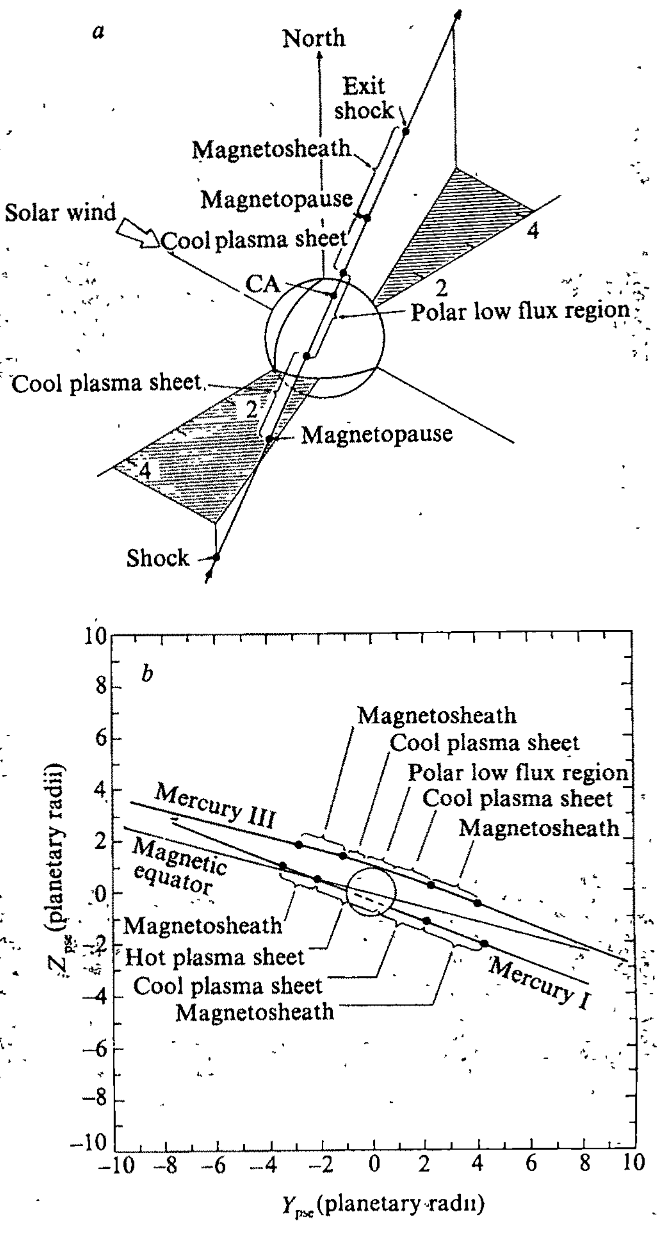
The results presented here are from an electron spectrometer mounted on a scan platform. It accepts electrons from the anti-solar direction in the energy range 13.4–690 eV in 15 logarithmically spaced channels of width $\Delta E/E = 6.6\%$. The instrument was mechanically scanned at a rate of 1° per second through an arc of 120° centred on a direction in the ecliptic plane 6° east of the spacecraft–Sun line. The field of view is fan shaped, ±13.5° in a plane containing the scan axis and ±3.5° in a plane perpendicular to the scan axis, which is maintained in the direction of Canopus by the attitude-control system of the spacecraft. The instrument obtains an electron spectrum every 6 s, from which the density, temperature and so on can be obtained[1].

Figure 1*a* shows an isometric view of the Mercury III trajectory and Fig. 1*b* a view from the Sun of the trajectories at both Mercury I and III, with the locations of observed plasma features marked. Entering and leaving the interaction region at each encounter, the plasma instrument detected the bow shock, the magnetospheric boundary and the intervening shocked solar-wind plasma (the magnetosheath). Within the magnetosphere, distinctive plasma electron populations were observed which exhibited large spatial and/or temporal variations. The magnetospheric populations observed at the two encounters differed significantly (Fig. 1*b*). Figure 2 shows electron fluxes in two energy channels for both encounters. Features labelled in Fig. 1 are identified here with vertical lines. The plasma signatures for the bow shock, magnetosheath and magnetospheric boundary seem to be similar in character at both encounters[1]. In the magnetosphere the Mercury I data show a transition between a cooler and a warmer electron population on the inbound and outbound trajectories. The Mercury III magnetospheric data show a sequence beginning with a cool plasma followed by a region of low fluxes in all energy channels and then returning to a cool electron population.

Spectra of the electrons from different plasma populations observed during Mercury III are shown in Fig. 3*a*, and a comparison with the "hot plasma sheet" spectra from Mercury I is made in Fig. 3*b*. The solar-wind spectrum exhibits the characteristic two-temperature profile. The expected increase in flux at all energies behind the bow shock is evident in the magnetosheath spectrum. The spectra of the "cool plasma sheet" populations measured at the two encounters are similar at energies above approximately 50 eV, but are quite different at lower energies; this difference is tentatively attributed to spacecraft charging. The plasma sheet is characterised by a flux above 100 eV which has a temperature even higher than that of the halo electrons in the solar wind, whereas the electrons in the low flux region have a temperature lower than those in the solar wind.

The magnetopause and bow shock were found in Mercury I to be geometrically similar to those of the Earth but reduced in size relative to the planet by a factor ~ 6. The present observations support this interpretation and provide further details of the plasma electron populations in Mercury's magnetosphere. Scaled up to the size of the Earth's magnetosphere, the Mercury I trajectory corresponds to a crossing of the Earth's magnetospheric tail from solar magnetospheric coordinates $X_{SM} \simeq -10R_e$ (Earth radii) and $Z_{SM} \simeq -3R_e$ on the dusk magnetopause to $X_{SM} \simeq -5R_e$ and $Z_{SM} \simeq 0R_e$ on the dawn magnetopause. The trajectory of Mercury III corresponds to a

Fig. 1. *a*, Isometric drawing of the trajectory of Mariner 10 during its third encounter with Mercury. Features of the interaction described in the text are shown along the trajectory. CA, Point of closest approach (327 km, at 2248:30 Earth-received time on March 16, 1975). *b*, View from the Sun of Mercury I and Mercury III, showing the approximate magnetic equator and the regions sampled during each encounter. The coordinate system is solar-ecliptic.



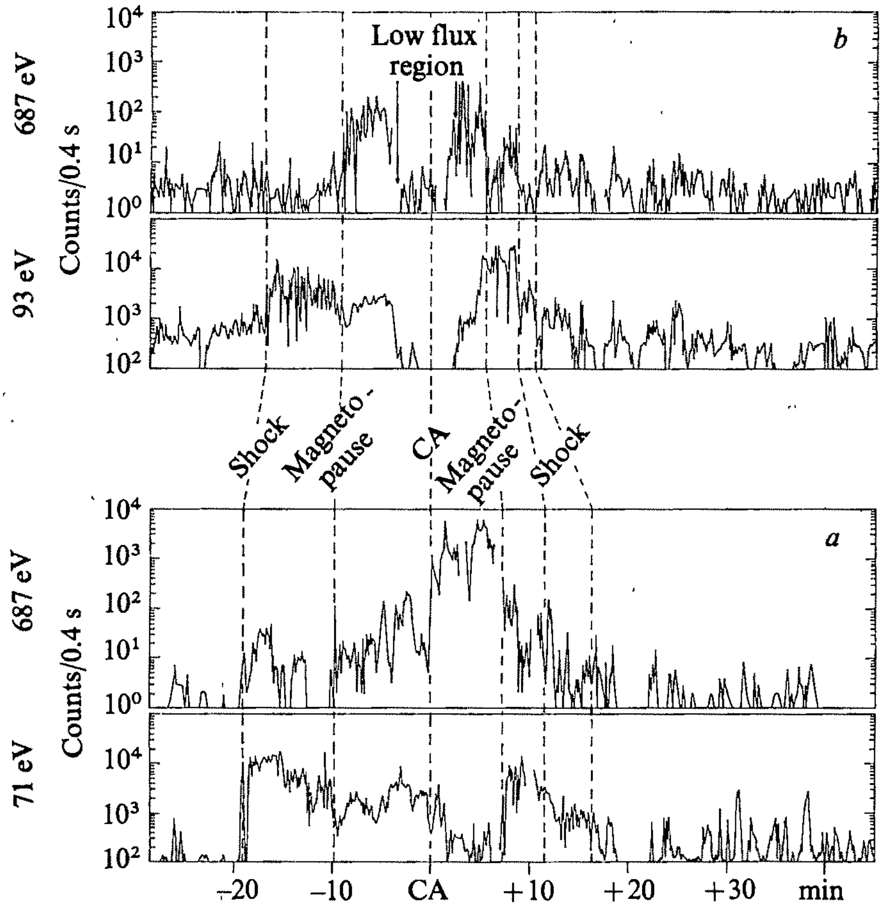


Fig. 2 Electron fluxes measured at 71 eV and 687 eV during Mercury I (*a*) and at 93 eV and 687 eV during Mercury III (*b*), with the bow shock, magnetopause and low flux region indicated by vertical lines. The curves have been aligned so that times of closest approach coincide.

similar crossing from $X_{SM} \simeq -6R_e$ and $Z_{SM} \simeq +4.5R_e$ to $X_{SM} \simeq 0R_e$ and $Z_{SM} \simeq +6R_e$. (The X_{SM} axis is the planet-Sun line. The Z_{SM} axis is positive northwards, defined such that the X_{SM}–Z_{SM} plane contains the dipole axis. These approximate solar magnetospheric coordinates are based on the Hermean magnetic dipole deduced from Mercury I magnetic field observations[2].) Both the present plasma observations and, in retrospect, those from Mercury I indicate large scale properties of the electron populations very similar to those which would be observed along scaled-up trajectories through the Earth's magnetosphere. The Earth's magnetospheric tail contains a plasma sheet centred about the solar magnetospheric (SM) equatorial plane and extending to a thickness of several R_e, characterised by a hot particle population (mean electron energy ≈ 1 keV) near the centre and a cool population (≈ 100–200 eV) near the edges; above and below the plasma sheet, on magnetic field lines connected to the polar caps, the particle intensities are very low. Figure 2 shows that all these regions have now been sampled at Mercury, at roughly the locations expected on the basis of scaling from Earth observations. The cool electron population observed at a relatively large distance from the SM equatorial plane by Mercury III is characteristic of the outer plasma sheet near the midnight meridian and at its largest distance from the SM equator, a region of low particle intensities which seems to be the analogue of the Earth's polar cap. At Mercury I, the spacecraft entered the magnetosphere at some distance from the SM equator and also observed a cool electron population, but on its outbound trajectory it was near the SM equator and observed a hot electron population similar to that in the central region of the Earth's plasma

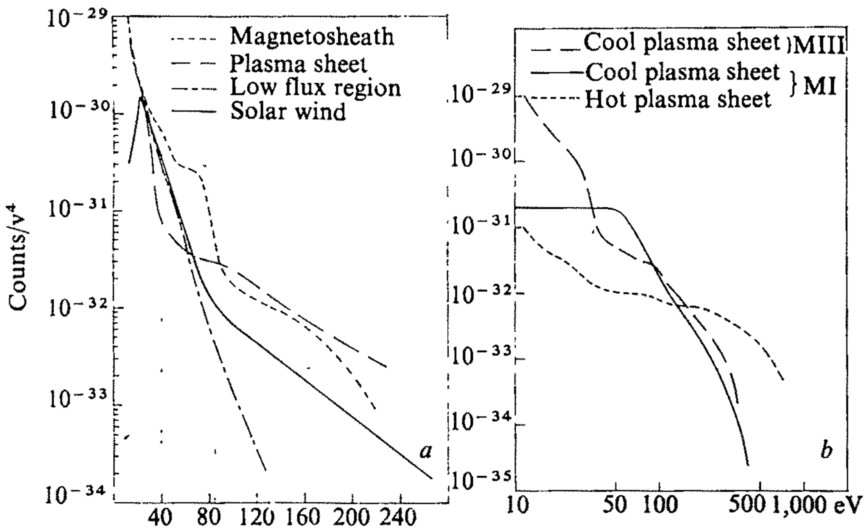


Fig. 3 *a*, Spectra of electrons observed at Mercury III. The vertical dotted line at 40 eV indicates distortion of the spectra below the energy as a result of spacecraft charging. The ordinate (counts/v^4) is proportional to the distribution function. *b*, Comparison between cool and hot plasma sheet spectra from the Mercury I observations with a cool plasma sheet spectrum from Mercury III.

sheet; as expected from its trajectory, it was never far enough from the SM equator to reach the low flux polar cap region.

The characteristic electron energy in the cool plasma sheet (Fig. 3*b*) observed in both Mercury I and III is $\sim$ 100–200 eV, whereas in the hot plasma sheet observed in Mercury I it is at least $\sim$ 1 keV; these energies are of the same order of magnitude as those observed in the corresponding regions of the Earth's plasma sheet. The fact that the plasma sheet energies in both magnetospheres are comparable implies that the plasma sheet energy does not scale with the electric potential difference across the magnetospheric tail, the value of which for Mercury should be about one-seventh of that for the Earth[3]; this has important implications for the still unsolved problem of the origin of the plasma sheet.

In summary, well defined bow shock and magnetospheric boundary crossings were observed on the inbound and outbound trajectories during Mercury I and III. The locations of the boundaries observed are mutually consistent with locations obtained from models which scale the Earth's magnetosphere to Mercury I data[1,4] and the other magnetospheric features are consistent with the magnetic field model[4]. Thus, the plasma results strongly support the idea that Mercury's magnetic field is intrinsic rather than induced, and show that the magnetosphere of Mercury is remarkably similar to that of the Earth.

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Possible identification of Ariel 1118−61

THE transient X-ray source Ariel 1118−61 has been discovered by Ariel V (refs 1, 2) and the best estimate for the position is (1950.0) $\alpha = 11$ h 18 min 59s, $\delta = -61° 35.3'$. We point out here the presence in the error box of the long period Mira-type variable RS Cen (1950.0) $\alpha = 11$ h 18 min 16.5 s, $\delta = -61° 36.0'$. It has been suggested that in the Mira-type variables o Ceti (Mira) and SY For, the blue variable companion is a white dwarf illuminated by infalling material accreted from the strong wind of the M-type star[3–5]. The similarity between these systems and the (recurrent) nova T CrB has also been stressed[6]. We propose that some transient X-ray sources are binary systems consisting of a compact object in orbit around a long period variable. The accretion rate of material on to the compact object in such a system[4] is $\lesssim 10^{-8}\ M_{\odot}\mathrm{yr}^{-1}$ which is just the rate required to produce X rays from a compact object. We note, too, the proposed identification[6] of another transient X-ray source (2U1543−47) with a variable late-type giant.

The large variability of radius and luminosity of a Mira-type star is likely to produce strong modulation of the stellar wind and a similar modulation of the accretion rate on to and thus luminosity of the compact object. A similar correlation between stellar wind strength and luminosity of the X-ray object has been proposed for Cen X-3 (refs 7, 8). We expect the modulation to be much stronger for Mira-type stars.

The transient source A1118−61 was observed to peak in mid-December 1974. The period of RS Cen is[9] 164.51 d and the X-ray source appeared at phase $\sim$ 0.4 (optical maxima occur at phase 0.0 and minima at 0.54) of the cycle when the radius of RS Cen is expected to reach a maximum. We intuitively expect mass outflow to be greatest near light minimum, when absorption bands are strongest in the stellar atmosphere. If the proposed identification is correct we expect the transient X-ray source to appear 'regularly' with a period of 164 d. Eclipses of the compact object by the red variable may complicate this prediction: the orbital period for such a system is $\gtrsim$ 2 yr. We would associate the 6.75 min periodicity of the X-ray object with the rotation (or possibly precession) period of the compact object[10].

The identification of A1118−61 with RS Cen would establish the identity of a new class of X-ray binaries. Such systems would offer a unique opportunity to study the structure and dynamics of the atmospheres and winds of Mira-type variables.

We thank Drs F. Rosenberg, P. Sanford and G. Skinner for communicating results in advance of publication.

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Observations of low energy gamma-ray bursts with SAS-2

Low energy celestial gamma ray bursts were discovered[1] in 1973 and the detection later confirmed by several groups. In this paper we report the low energy gamma-ray bursts observed by the plastic scintillator anticoincidence dome of the Small Astronomy Satellite-2 (SAS-2) gamma-ray telescope. Although the high energy gamma-ray telescope of SAS-2 has a threshold of about 20 MeV, the large SAS-2 anticoincidence dome (A-dome), which is the outermost element of the instrument, provides a very large detector for the high energy portion ($\geqslant$0.3 MeV) of these burst events.

Details of the instrument are given by Derdeyn *et al.*[2]. The A-dome, constructed out of 1.5 cm thick scintillator has an average collection area of about 2.5×10^3 cm^2 and is an almost omnidirectional detector, only being insensitive to directions within a small cone ($\sim$30° half angle) centred around the direction that it is attached to the spacecraft. The average effective threshold for detection over the dome is about 0.15 MeV, the average efficiency rising to about 20% at 0.6 MeV. The counting rate of the A-dome is accumulated and read out every 768 ms. When there is no increase resulting from the trapped radiation in the Atlantic anomaly, the observed rate is about 4.2×10^3 counts s^{-1} and it remains quite steady. (The Earth's trapped radiation extends downward to unusually low altitudes in the central

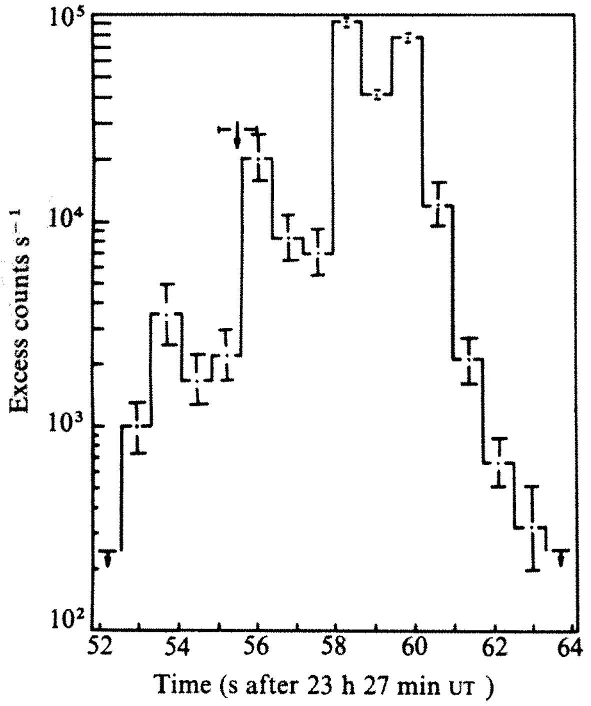

Fig. 1 Time history of the low energy gamma-ray burst observed in the SAS-2 A-dome on March 2, 1973. The arrow shows the event time observed by the Vela satellites. The counting rates shown are the excess counts above the steady SAS-2 background of 4.2×10^3 counts s^{-1}.

and south Atlantic. These low energy electrons and protons cause an increase in the count rate of the A-dome.)

SAS-2 was launched on November 15, 1972 into an equatorial orbit with a 440-km perigee and 610-km apogee. During the period of experimental operation (November 20, 1972–June 8, 1973, the counting rate from the A-dome provided a continuous monitor for fluctuations in the low energy gamma-ray fluxes from the unocculted sky. Using these data a search has been made for sudden rate increases where there is an excess of 600 counts or more over the background in at least three successive 0.768 s readouts. Although this criterion would not pick up events with time scales shorter than 2 s, the occasional noise spikes in single readouts made its adoption necessary. The selection criterion is further biased against events which last longer than 30 s because of background comparisons which were made on this time scale.

This procedure resulted in the detection of two events observed by other satellites and the discovery of one, which has now been confirmed by other satellite observations. In addition, two events on the list of those detected by the Vela satellite series[3] were not seen by SAS-2. In view of the high sensitivity of SAS-2, these negative results suggest that the Earth occulted the source, as it will, on average, 40% of the time. Table 1 lists the five events that have been confirmed as multisatellite observations. There was no reported solar activity during any of these events.

Considering the two events not seen by SAS-2, if it is assumed that the absence of a detectable signal in the SAS-2 A-dome is an Earth occulation effect rather than a large variation in the expected energy distribution, then the regions of the sky in which the events occurred may be restricted regions. These are a 70° cone about $\alpha=36.6°$, $\delta=1.0°$ in the case of the December 18, 1972, event and a similar cone about $\alpha=27.4°$, $\delta=1.6°$ in the case of the May 7, 1973, event. For the December 18, 1972, event this cone is consistent with the location of the source lying in the region $70°<\alpha<120°$, $0°<\delta<20°$ as determined by Imhof *et al.*[4] except that it excludes the highest part of the α range. The time history of the March 2, 1973, event as measured by SAS-2 is shown in Fig. 1. Notice that the time given by the Vela satellites for the first detection of an increase is well into the event. These data support the point of view that the gamma-ray bursts observed have a complex time structure and may have durations in excess of those measured by Vela type detectors that trigger on sudden increases[4].

Even with the SAS-2 time resolution, four distinct peaks appear indicating a significant temporal structure (Fig. 1). The event reaches a peak intensity of 9.4×10^4 counts s^{-1}, more than two orders of magnitude greater than the threshold of 7×10^2 counts s^{-1} which the event must surpass in the 0.768 s readout interval in order to be selected in the computer scan of the SAS-2 data. Considering the occultation of SAS-2 by the Earth, the event must have occurred in a region of the sky within a cone of 110° around $\alpha=49.4°$, $\delta=0.1$. The Vela data (R. W. Klebesadel, R. A. Olsen and I. B. Strong, private communication) indicate the source was in a circular band of radius $82.5\pm4°$ centred at $\alpha=193.9°$, $\delta=25.7°$.

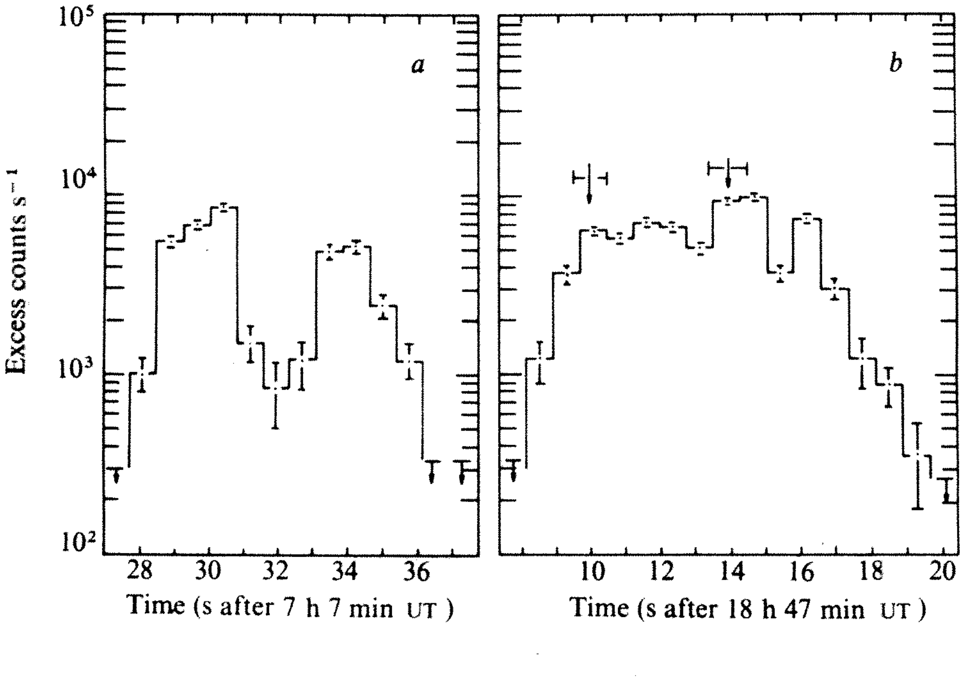

Fig. 2 Time histories of two gamma-ray burst events seen by the SAS-2 A-dome on June 6, 1973. The arrows show the event times observed by the Vela satellites. *a*, Vela 5A; *b*, Vela 6A. The counting rates shown are the excess counts above the steady SAS-2 background of 4.2×10^3 counts s^{-1}.

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Two events seen by SAS-2 occurred on the same day, June 6, 1974, which is surprising considering the average event rate. The time profiles of these two events are shown in Fig. 2. There were only 12 detected cases in the approximately 6.5 months that the A-dome rate exceeded the threshold level and remained above background (by $>6\times10^2$ counts s^{-1}) for more than three readout intervals (approximately 2.3 s) except when the satellite passed through the Atlantic trapped radiation anomaly. The second June 6 event was subsequently confirmed by the IMP-7 satellite[5] and the Vela system (Table 1). Notice that again for the June 6 event which did trigger the Vela system the Vela time for first detection occurs after the event has started and reached a level almost an order of magnitude above the SAS-2 threshold. Considering the occultation of SAS-2 by the Earth for the two June 6, 1973, events, the first must have occurred within 110° of $\alpha=184.1°$, $\delta=1.2°$ and the second within 110° of $\alpha=317.7°$, $\delta=-1.9°$. The other events observed by SAS-2, but not confirmed by other satellites, seem to be genuine events although the estimated fluxes are in the range of 3×10^{-8} to 10^{-5} erg cm^{-2} which may reduce the probability of detection by the other satellites involved.

Table 1 Confirmed multisatellite observations (November 20, 1972–June 8, 1973)

Date	Time h min s	Satellite and reference
December 18, 1972	20 27 39	2 Velas*; IMP-7[5]; 1972-076B[4]
March 2, 1973	23 27 53	2 Velas*; IMP-7[5]; SAS-2
May 7, 1973	08 04 32	3 Velas[2]
June 6, 1973	07 07 28	IMP-7[5]; SAS-2
June 6, 1973	18 47 08	SAS-2; IMP-7[5]; 2 Velas*

The satellite listed first is the one from which data first led to recognition of the event.

*Unpublished results from R. W. Klebesadel *et al.*

In view of the significant sensitivity of SAS-2 as suggested by these comparisons and direction calculations, it may seem surprising that no other events with timescales betwen 2 and 30 s were observed; however, it is difficult to draw quantitative conclusions from this result because of the difficulty of comparing results from detectors with vastly different characteristics.

In none of the events was there any significant excess of high energy gamma rays in the spark chamber. This is not surprising since with the 0.25 sr solid angle of the high energy telescope, there is less than a 5% chance that an event will fall within the telescope acceptance angle even if there is a detectable flux of gamma rays with energies above 25 MeV.

We thank Drs Klebesadel, Strong and Olsen for providing unpublished Vela data and Drs Cline and Desai for reviewing the IMP-7 data to determine the existence of events at the times suggested by the SAS-2 data. H. Ö. is an NRC Senior Postdoctoral Associate.

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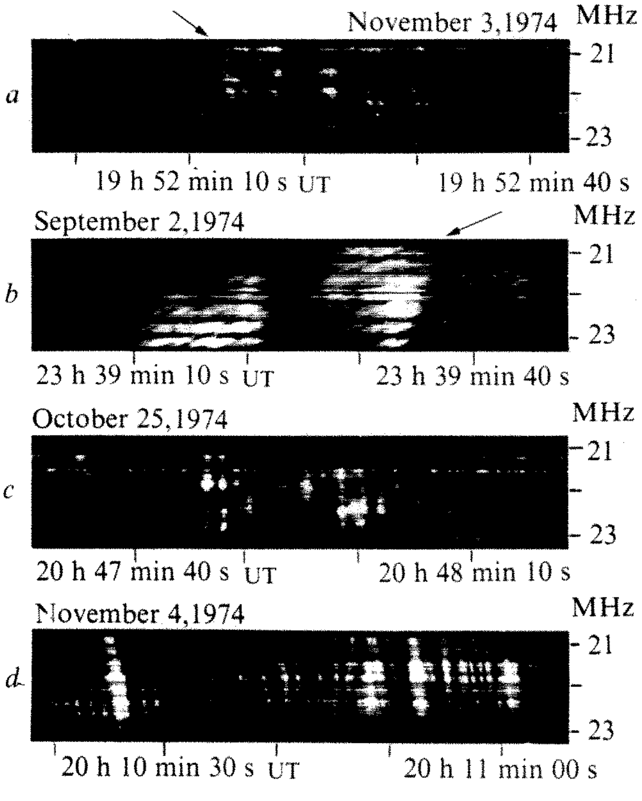
Polarisation structure of Jupiter's decametre radio bursts

THE high-resolution dynamic spectra of Jupiter's decameter radio emission exhibit structures consisting of repeated, tilted ridges[1]. The frequency–time slope of these 'diagonal patterns', also referred to as modulation lanes, varies from 50 to 300 kHz s^{-1}. The slope is a function of the System III central meridian longitude of Jupiter, and it may be either positive or negative[2,3]. Recent observations of modulation lanes are described by Ellis[4,5] and Lecacheux[6]. Here I report a study of polarisation diversity in the spectra of modulation lanes.

The frequency range 21–23 MHz is close to the region above which the Jovian bursts are predominantly elliptically polarised in the right hand (RH) sense, but below which the proportion of bursts polarised in the left hand (LH) sense increases. Since most of the spectra of the lanes have been recorded in RH circular or linear polarisation, no conclusive information on the relationship of the modulation lanes to polarisation is available[7].

Rapid reversals of the sense of polarisation have previously been observed in spectral records of groups of S bursts[8]. The phenomenon occurred as a function of time and frequency, and was referred to as 'polarisation diversity'. This polarisation diversity has been observed[9-12] in fixed frequency records of L-bursts. The reversals of polarisation sense were often cyclic with time. To study the function of diversity in modulation lanes, the spectra of the RH and LH circular components must be recorded separately, as in the experiment reported here.

Fig. 1 Samples of high-resolution spectra of Jupiter's decametre radio bursts. Modulation lanes are shown in *a* (positive drift) and *b* (negative drift) indicated by arrows. The durations of the envelopes are long in *b* but short in *c*. The envelopes seem to be tilted in *d*. The wide, parallel horizontal lanes are residual Faraday fringes. In *b* the antenna polarisation was linear and the Faraday fringes seem to be very strong.



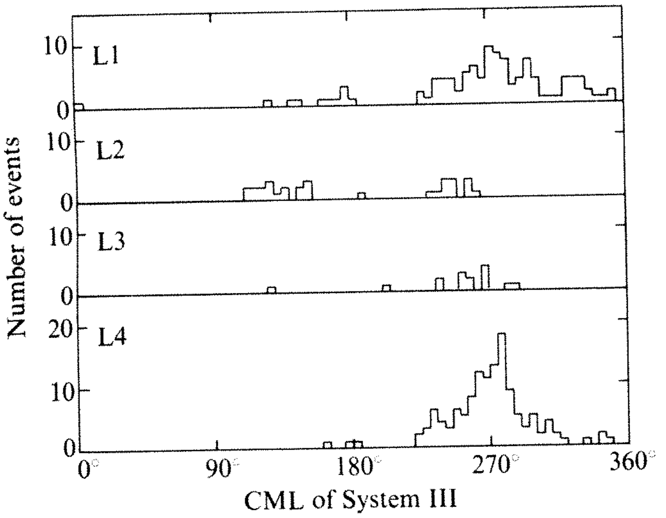


Fig. 2 Number of events of types L1 to L4 as a function of System III (1957.0) central meridian longitude (CML).

A new instrument was built for this purpose, the antenna of which is a steerable system of two cross log-periodic units in parallel. The circular components are obtainable with broadband quadrature hybrid circuits. The main equipment is a 48-channel receiver for the range 20.85–23.10 MHz. The channels are separated by intervals of 50 kHz and have 3 dB bandwidths of 15 kHz, with a signal integration time constant of 20 ms. The display unit consists of a two-colour (red and green) array of 96 light-emitting diodes, switched at a rate of 1 kHz in synchrony with the radio frequency switch at the output ports of the hybrid circuits.

The sense of polarisation is indicated by the colour of the spectrum, since colour is the only extra dimension available in an intensity-modulated frequency–time representation. The recording is made on a 16 mm colour reversal film. If the emission is highly elliptical, the amplitudes of the RH and LH components are almost equal, and the resultant colour is yellow. For highly circular emissions in the RH or LH sense, the spectrum appears green or red, respectively. Slight variations in colour can be detected faster and with greater confidence than small density differences in two parallel black-and-white records.

The observations were carried out from August 1 to November 17, 1974, for 2 h each night at Kiiminki, 30 km east of Oulu. Other spectrographs of the sweep-frequency type were operated simultaneously. Only the multichannel observations are described here. Some interference was caused by residual Faraday fringes. Because of the relatively low altitude of Jupiter (15–18°), the effect of ground reflection could not be avoided. Thus the Faraday rotation modulated the axial ratio of the polarisation ellipse and the relative amplitudes of the circular components.

Sample records reproduced in black-and-white are shown in Fig. 1. Prominent features in the spectra are the radiation areas (envelopes). The modulation lanes traverse them diagonally in a continuous manner. According to the classification given previously[2], *a* and *b* represent bursts of types L2 and L4, respectively. In the same notation L1 indicates a burst with no detectable lane structure, and L3 a burst in which the positive and negative lanes are superposed.

The durations of the envelopes are related to the elongation of the planet; towards opposition the durations tend to increase[3]. Accordingly, the duration of envelopes in Fig. 1*b* are longer than those in Fig. 1*c*. 'Fast-drift lanes' are observed occasionally, with frequency–time slopes an order of magnitude greater than those of the modulation lanes (Fig. 1*d*). Similar events have been recorded by Ellis[5,13].

An occurrence histogram of bursts of types L1 to L4 is presented in Fig. 2. This is constructed in the same way as that for the 1966–69 observations[3]. Again, type L4 bursts are associated chiefly with regions A and C, and type L2 bursts with region B. But the number of L1 bursts relative to bursts of other types is greater than before, the number of L2 and L3 bursts is less, and bursts of type L3 occur chiefly at region A rather than at region B. The region designation is that of Carr, *et al.*[14].

The result of a careful search for polarisation diversity effects indicates that diversity does not occur as a rule in the spectra of modulation lanes. Most lanes are simple variations in intensity of emission, and thus are similar in appearance in both circular modes. The polarisation is elliptical in the RH sense and the amplitude of the RH component is the larger of the two.

There are a few cases in which diversity does appear. The sense of polarisation is then LH, and may be recognised easily from the colour. Polarisation diversity seems to occur in areas where the amplitude pattern of the spectrum is more complex than just a simple set of lanes. In two such spectra obtained it seems that the LH component may belong to one of the two sets of lanes of the L3 burst. Not all of the L3 bursts, however, display polarisation diversity.

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Received March 10; accepted April 11, 1975.

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Wear of synthetic diamond when grinding ferrous metals

It is well established that diamond is an inefficient abrasive for grinding ferrous materials, especially the low carbon variety. The widely held view is that as ordinary carbon has a strong affinity for iron, forming iron carbide (Fe_3C), this should also be the case with diamond (cubic carbon) when grinding steel. The high rate at which diamond grinding wheels wear down when grinding low carbon steel is said to be a consequence of this action. We suggest here another mechanism, evidence of which is provided by scanning electron micrographic (SEM) data.

To simulate grinding and at the same time eliminate the influence of the bonding material, cutting tests have been performed using a single diamond particle mounted on the periphery of a 20 cm (8 inch) diameter aluminium disk. A groove was cut in a soft, low carbon, steel plate (AISI 1018 steel) by moving the flat plate into the rotating diamond particle at a speed v. This method of simulating the action of an abrasive particle in the surface of a grinding wheel is termed overcut fly milling[1,2]. The machine settings—table speed, v: 30 cms^{-1} (12 inches min^{-1}); downfeed (depth of groove), d: 25 μm (0.001 inch); and disk speed, V: 20.3 ms^{-1} (4,000 feet min^{-1})—were chosen to give a chip size comparable to that used in fine surface grinding using a diamond grinding wheel (resulting in an undeformed chip thickness of a few microns). A well developed, single crystal, synthetic (cubo-octahedral) diamond was chosen for study. This type of diamond was

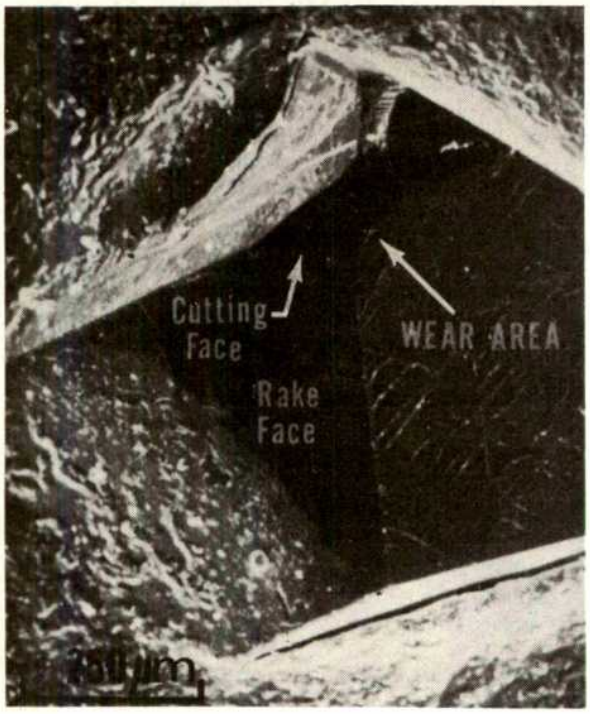


Fig. 1 Micrograph of the wear area of a diamond grit.

selected in preference to a polycrystalline specimen because it is considerably stronger and less inclined to chip by intercrystalline fracture. This combination of tool and method precluded diamond wear by chipping.

Several thousand cuts were made on the work material with each diamond particle tested. After a test, the worn diamond surface was coated with a thin layer of gold–palladium (to overcome the charging problems otherwise experienced in the scanning electron microscope) and the wear areas were examined.

Figure 1 is a micrograph of the worn area of a diamond grit examined in the secondary electron mode. Figure 2 is a micrograph of the worn area obtained by tilting the sample favourably and examining it in the back scatter mode. The worn area is not plane but has a concave shape in a direction transverse to the direction of cut. Figure 3 shows the groove-like markings evident in Fig. 2 at higher magnification. These markings result from a transverse saw tooth pattern (Fig. 4). No wear was evident on the rake face of the diamond across which the chip flows under high pressure.

All of the results described here are consistent with a mechanism of wear in which diamond is converted to graphite before its removal from the worn surface.

Diamond at room temperature is in a metastable state and will transform to graphite in the proper circumstances. An increase of temperature without a suitable increase in pressure will carry the diamond further from equilibrium and the presence of a properly directed shear stress will promote further the transformation to graphite. The presence of a material that rapidly removes graphite from the surface of the diamond should promote this transformation even more. Hot, low carbon steel is such a material. This latter situation does not arise when grinding aluminium or copper alloys with diamond and, consequently, the rate of wear is negligible in those circumstances.

All of these conditions (high temperature, relatively low pressure, high shear stress and hot iron) are present on the worn area on the clearance face of a diamond abrasive particle that is cutting soft, low carbon steel.

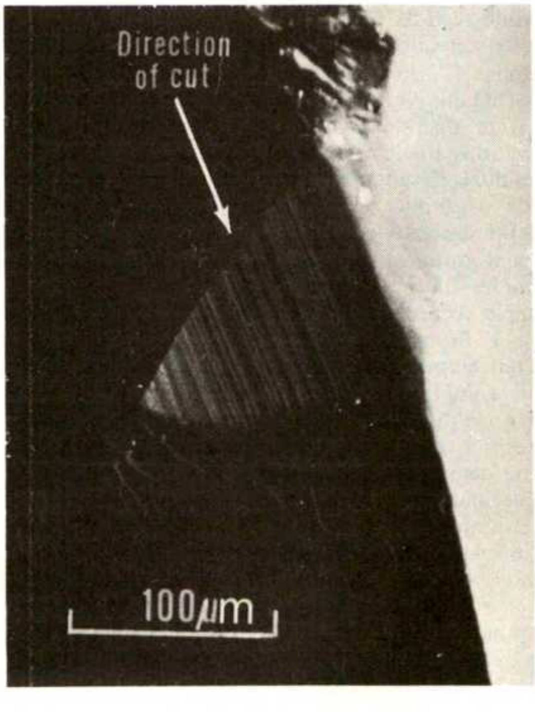
The fact that diamond (unlike graphite) is relatively insoluble even in molten iron (a key point in the synthesis of diamond[3]) rules out the possibility that diamond reacts directly with iron.

Once diamond graphitises, however, the graphite so formed can rapidly diffuse into iron because of the intimate contact under pressure, the high affinity of graphite for iron and the high interfacial temperatures. Also, the fact that diamond is worn more rapidly by a soft, low carbon steel than it is by a strong, highly alloyed steel, tends to rule out oxidation as the major cause of diamond wear since the alloy steel would give rise to a higher grinding temperature.

The grooves evident in Figs. 2 and 3 could not have been produced by direct abrasive action of any constituent in the soft steel being ground. Instead, those grooves must result from a preferential transformation on 110 planes, as shown in Fig. 4. Prins *et al.*[4] have reported similar markings on diamonds used to grind hardened tool steel (AISI M42) but were unable to explain their presence. Experiments conducted by Seal[5] has shown that the 001 faces of diamond were the most resistant to high temperature thermal graphitisation whereas the 110 planes were the least resistant. Bovenkerk *et al.*[6] have reported that diamonds grow with cubic habit when the conditions of temperature and pressure are almost minimal for a particular catalytic-solvent material, but that they grow with octahedral habit when the temperature and pressure are very much higher. As Seal[5] has pointed out, the directions of fastest graphitisation are the same as those of the fastest growth, and the directions of slowest graphitisation coincide with those of slowest growth in the lower pressure part of the region of diamond synthesis. These striking similarities suggest strongly that the catalytic-solvent effect of iron, and the concomitant conditions of high temperature and lower contact pressures (relative to the stable region of the diamond) when grinding low carbon steel with diamond, play a very significant role by causing rapid, preferential graphitisation of diamond, leading to poor grinding performance.

The sharp lines evident in Figs 2 and 3 on the worn areas are approximately parallel but do change direction and intersect occasionally. These lines are thought to be the ends of what Tolansky[7] refers to as slip planes (found in man made diamonds) which are akin to the foliation markings in natural stone that has been subjected to plastic flow under enormous pressure. It is of interest to note that the spacing of these slip

Fig. 2 Micrograph of the same wear area obtained by tilting the sample and examining in the back-scatter mode.



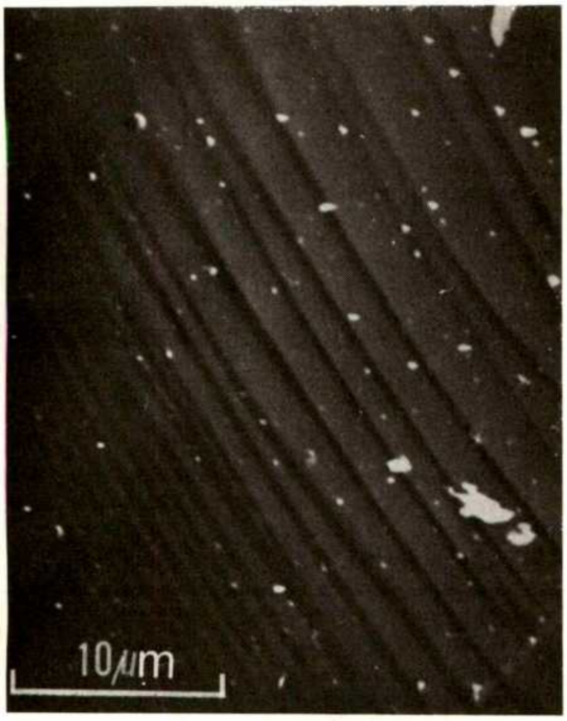


Fig. 3 Groove-like markings in the wear area of diamond grit.

planes is from 1 to 3 µm in the samples of diamond used in this investigation.

The concave shape of the general wear surface is also consistent with the graphitisation theory of wear presented here. The temperature in the central region of the cut will be higher than at the edges and this should give rise to a higher rate of graphitisation in the former region.

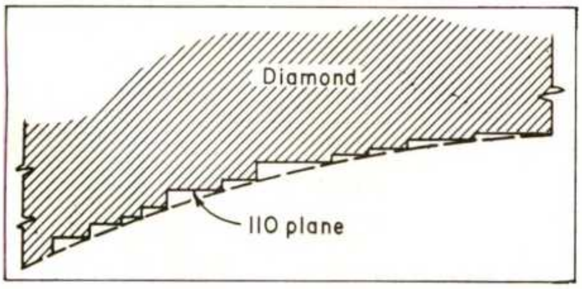


Fig. 4 Graphical representation of saw-tooth markings shown in Fig. 3.

This work has been sponsored by a grant from the National Science Foundation. We thank Dr T. J. Clark, Specialty Materials Department of the General Electric Company of USA, for providing the single crystal diamonds used in this study.

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Received March 21; accepted April 8, 1975.

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Chemistry and structure of high pressure phase of garnets rich in almandite

I HAVE previously reported[1,2] high pressure phase transformations for garnets of the almandite–pyrope ($Fe_3Al_2Si_3O_{12}$–$Mg_3Al_2Si_3O_{12}$). Here, the chemical formula and the crystal structure for the high pressure phase of almandite-rich garnets quenched from a loading pressure of approximately 120×10^5 kPa and temperatures of about 1,400–1,800 °C are given.

Details of the samples and experimental procedures have been described elsewhere[3,4]. Fine polycrystalline powder samples of synthetic almandite and natural almandite-rich garnets were compressed in a diamond anvil press fitted with a lever and spring assembly and were irradiated by a continuous wave YAG laser. The loading pressures of the sample at the central portion of the anvil were estimated from the length of the spring which was calibrated according to the room temperature NaCl pressure scale. The real pressure to which the sample was subjected is probably $\sim 40 \times 10^5$ kPa greater than the loading because of a transient increase of pressure during heating. The temperatures of the samples could be estimated using an optical pyrometer but the uncertainties would be rather large.

The quenched samples have been X rayed with CoKα radiation in a modified 57.3-mm Debye–Scherrer camera. The resultant diffraction data have been listed in Tables 1 and 2 for the synthetic almandite–garnet and the natural almandite-rich garnet. With the exception of the reflection at $d = 2.95$ Å, nearly 40 observed d-spacings for the high pressure product from synthetic almandite can be indexed on the basis of a structure isotypic with the hydrogarnet, griphite[5,6]. Many reflections such as 2.866, 2.559, 2.444 Å . . . , which seemed initially to be those of the starting material, almandite, can equally well be derived from the new high pressure phase. This is evident from comparison of the observed d-spacings with those calculated for almandite and for the griphite isotype. The calculated lattice parameter for the high pressure phase with the griphite structure is $a_0 = 11.470 \pm 0.025$ Å. The space group may be $P2_1/a\bar{3}$ (ref. 6). The reflection of 2.95 Å may correspond to the most intense reflection (110) of stishovite. Thus, almandite seems to disproportionate into a mixture of stishovite plus a phase with the griphite structure at approximately 120×10^5 kPa loading pressure and 1,400–1,800 °C. With the exception of a very weak unidentified line, 2.071 Å, the high pressure phases for the natural almandite-rich garnet (Table 2) are almost identical to those of the synthetic almandite with the lattice parameter for the griphite structure $a_0 = 11.465 \pm 0.030$ Å.

Griphite is a hydrophosphate garnetoid. Its chemical formula represents a complex example of a general form of $X_3Y_2(ZO_4)_{3-m}(OH)_{4m}$ with $m = 0.5$ (ref. 5). Because no water was involved in this study of phase transformations of garnet[2], one would expect the general formula for hydrogarnets to be modified to suit the possible chemical formula of the high pressure phase of almandites. Removing water completely, $2m(H_2O)$, from the general form of hydrogarnet yields another general formula of $X_3Y_2(ZO_4)_{3-m}(O)_{2m}$. If the high pressure phase with griphite structure has this formula, then the high pressure disproportionation of almandite can be represented as

$$\underset{\text{garnet}}{Fe_3Al_2(SiO_4)_3} > \underset{\text{griphite}}{Fe_3Al_2(SiO_4)_{3-m}(O)_{2m}} + \underset{\text{stishovite}}{mSiO_2}$$

Stishovite is indicated by the diffraction patterns (Tables 1 and 2), confirming the proposed reaction. The precise value for

Table 1 Room temperature and 1 bar pressure X-ray diffraction data of synthetic almandite quenched from a loading pressure of approximately 120×10^5 kPa and 1,400–1,800 °C (CoKα).

Observed		Almandite		Griphite			Stishovite§	
I/I_{100}*	*d*(Å)	*d* (Å)†	*hkl*	*d* (Å)‡	*hkl*	*d* (Å)	*hkl*	I/I_{100}
20	4.67			4.68	211			
5	3.47			3.46	311			
5	3.33			3.31	222			
15	3.07			3.07	321			
15	2.95					2.95	110	100
80	2.866	2.883	400	2.868	400			
?	2.78			2.78	410,322			
100	2.559	2.579	420	2.565	420			
40	2.444	2.459	332	2.445	332			
50	2.341	2.354	422	2.341	422			
40	2.247	2.262	510	2.249	510,431	2.24	101	30
40	2.210			2.207	511,333			
50	2.090	2.105	521	2.094	521	2.09	200	1
25	2.020	2.039	440	2.028	440			
—	—					1.98	111	40
5	1.936			1.939	531			
15	1.915			1.912	600,442			
50	1.860	1.871	611	1.861	611,532	1.87	210	20
5	1.815			1.814	620			
?	1.72			1.729	622			
40	1.656	1.665	444	1.656	444			
10	1.622			1.622	710,543			
70	1.590	1.599	640	1.591	640			
15	1.561			1.561	721,633			
80	1.533	1.541	642	1.533	642	1.530	211	70
—	—					1.476	220	30
30	1.435	1.442	800	1.434	800			
10	1.417			1.423	810			
				1.412	811			
5	1.396			1.401	733			
				1.391	820			
15	1.361			1.371	653			
				1.352	822,660			
—	—					1.333	002	10
—	—					1.322	310	5
30	1.281	1.289	840	1.282	840			
40	1.253	1.258	842	1.252	842			
—	—					1.235	301	25
25	1.223	1.229	664	1.223	664			
5	1.212			1.216	922			
				1.209	930	1.215	112	10
15	1.159	1.165	941	1.159	941	1.159	320	10
5	1.126	1.131	862	1.125	862			
30	1.0678	1.0707	864	1.0696	953			
				1.0650	864			
30	1.0500	1.0527	10,4,2	1.0471	10,4,2			
20	1.0173	1.0193	880	1.0138	880			
—	—					0.9900	222	5
10	0.9608	0.9610	12,0,0	0.9625	965			
20	0.9480	0.9479	12,2,0	0.9493	12,1,1			
				0.9460	11,5,1			
30	0.9356	0.9354	12,2,2	0.9365	11,5,2			

*Estimated visually. The question mark denotes extremely weak lines.

†Observed in a standard Debye–Scherrer film with the exception of the last three reflections. *d*-spacings were calculated on the basis of $a_0 = 11.532$ Å reported by Takahashi and Liu[3].

‡Calculated from $a_0 = 11.470$ Å (see text).

§From Chao *et al.*[7].

m is not known at present, but the maximum value can be calculated since

$$\underset{(115.45\ \mathrm{cm^3\,mol^{-1}})}{V^0\text{almandite}} \geqslant \underset{(113.60\ \mathrm{cm^3\,mol^{-1}})}{V^0\text{griphite}} + m \underset{(14.03\ \mathrm{cm^3\,mol^{-1}})}{V^0\text{stishovite}}$$

where V^0 is the standard molar volume. So $m \leqslant 0.13$. The observed relative intensities for griphite and stishovite in Tables 1 and 2 support this estimate. If $m = 0.10$, the volume change associated with the disproportionation of almandite is -0.4%.

The quantity of stishovite in the products is expected to be very small, the evidence of its presence is rather weak since only one reflection line has been found to correspond to the most intense line of stishovite. An alternative explanation would be that this weak line (2.95 Å) may be derived from one of the intermediate phases between the griphite and the perovskite structures[1,2]. The observed griphite form may, therefore, be a stoichiometric $X_3Y_2(ZO_4)_3$ compound with the associated volume change for the polymorphic phase transition being -1.6%.

The high pressure phase of griphite structure found in this study is probably formed within a pressure range of 100–150×10^5 kPa. This pressure can be created naturally by meteorite impact, which seems to be the origin of mineral stishovite. Rocks containing a high proportion of almandite, such as mica and other schists, that have been subjected to

Table 2 As Table 1, for natural almandite*

Observed		Natural almandite		Griphite		Stishovite		
I/I_{100}	*d* (Å)	*d* (Å)	*hkl*	*d* (Å)	*hkl*	*d* (Å)	*hkl*	I/I_{100}
15	4.70			4.68	211			
15	3.45			3.46	311			
10	3.23			{3.31	222			
				3.18	320			
10	3.06			3.06	321			
15	2.96					2.95	110	100
80	2.864	2.881	400	2.866	400			
5	2.80			2.78	410,322			
100	2.564	2.577	420	2.564	420			
40	2.445	2.457	332	2.444	332			
50	2.342	2.352	422	2.340	422			
45	2.247	2.260	510	2.248	510,431	2.24	101	30
40	2.210			2.206	511,333			
45	2.095	2.104	521	2.093	521	2.09	200	1
5	2.071†							
20	2.030	2.037	440	2.027	440			
?	1.97			1.966	530	1.98	111	40
50	1.861	1.869	611	1.860	611,532	1.87	210	20
15	1.814			1.813	620			
15	1.732			{1.748	533			
				1.728	622			
40	1.657	1.663	444	1.655	444			
5	1.621			1.621	710,543			
70	1.592	1.599	640	1.590	640			
10	1.564			1.560	721,633			
80	1.534	1.540	642	1.532	642	1.530	211	70
—	—					1.476	220	30
30	1.436	1.440	800	1.433	800			
10	1.416			{1.422	810			
				1.411	811			
5	1.395			{1.401	733			
				1.390	820			
15	1.368			1.370	653			
—	—					1.333	002	10
—	—					1.322	310	5
25	1.283	1.288	840	1.282	840			
30	1.253	1.257	842	1.251	842			
—	—					1.235	301	25
20	1.224			1.222	664			
10	1.210			1.209	930	1.215	112	10
15	1.160	1.164	941	1.158	941	1.159	320	10
10	1.125	1.130	862	1.124	862			
40	1.0676	1.0699	864	{1.0691	953			
				1.0645	864			
30	1.0500	1.0519	10,4,2	{1.0554	10,3,3			
				1.0466	10,4,2			
30	1.0172	1.0185	880	{1.0214	10,5,1			
				1.0134	880			
—	—					0.9900	222	5
10	0.9596	0.9603	12,0,0	{0.9621	965			
				0.9554	12,0,0			
15	0.9476	0.9472	12,2,0	{0.9489	12,1,1			
				0.9456	11,5,1			
20	0.9347	0.9346	12,2,2	0.9361	11,5,2			

*See footnotes of Table 1 with the exception that the *d*-spacings for natural almandite were calculated from $a_0 = 11.523$ Å reported by Takahashi and Liu[3] and those for griphite structure from $a_0 = 11.465$ Å.

†Unidentified line.

impact may yield minor quantities of the new griphite phase.

I thank A. F. Reid and A. E. Ringwood for comments. The laser work was supported by a NSF grant while I was at the University of Rochester.

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Received February 11; accepted April 3, 1975.

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Benthic nutrient regeneration and its coupling to primary productivity in coastal waters

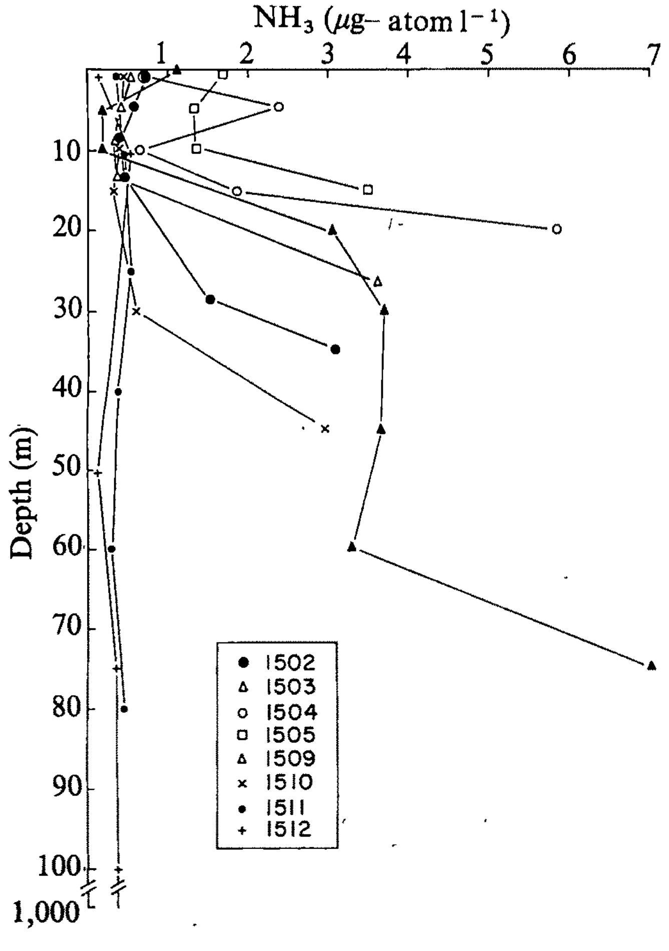
SHALLOW, near-shore ocean waters support high primary production because of the availability of inorganic nutrients. The availability is usually attributed to the proximity of freshwater runoff or to coastal upwelling and deep water advection[1,2].

The effects of nutrient regeneration from the ocean bottom have not, however, been estimated. We report here the first successful attempts to measure directly the nutrient flux from nearshore sediments. We contend that the continental shelf itself is a tightly coupled component of most near-shore ecosystems and that the function of the shallow sea floor in nutrient regeneration has been underestimated in previous investigations.

Nitrogen is considered to be the element limiting photosynthesis in coastal waters[3]. Although it has been presumed that the bottom is an important site of nitrogen remineralisation[4], attempts to estimate benthic regeneration have been confined to lakes[5,6], indirect inferences from concentrations[7,8] of pore water, or laboratory incubations which indicate that the bottom is only a minor source of nutrients[9] or even a nitrogen sink in anoxic areas[10].

It is necessary to know the rate at which ammonia and nitrate diffuse into the water column from the sediment to determine the degree of coupling between the bottom and primary production in the water. An indirect method which we have used is the assumption that the breakdown of organic matter in sediment is proportional to the amount of oxygen required by the sediment[11,12] although that probably, to some degree, underestimates total catabolism[13]. For each millilitre of oxygen consumed, we assumed that 0.412 mg organic carbon was oxidised to CO_2. The organic carbon–nitrogen ratio in sediments is about 10:1, or greater, and therefore we assumed that 0.041 mg organic nitrogen would be remineralised to ammonia for each millilitre of O_2 consumed. Numerous measurements of the amount of oxygen required by the sediment have been made and it is, therefore, relatively easy to estimate the degree of ammonia production of the bottom in many regions. The oxygen demand of the sediment in the sublittoral zone ranges from about 1 to over 100 ml $m^{-2} h^{-1}$ (ref. 12) and, therefore, 0.041–4.1 mg NH_4 $m^{-2} h^{-1}$ (2.0–292 μg-atom NH_4) should be produced by excretion.

Fig. 1 Concentrations of ammonia in the water column at selected locations on the continental shelf off New York, September, 1970 (ref. 3 and N. Corwin, unpublished). Stations 1504 and 1505 are the New York City sewage sludge dump site and the Army Corps of Engineers dredge spoils dump site, respectively. The terminal datum point on each profile is from the bottle, several metres above the bottom.



We presumed that if the predicted regeneration occurred it would diffuse out of the bottom. We therefore carried out *in situ* experiments to test this. An opaque, 7.5-l bell jar, with a stirring motor and an O_2 electrode[14], was used to incubate a 730 cm^2 area of the bottom for various lengths of time. (The stirrer circulates the water at a velocity somewhat less than natural tidal currents—up to 15 cm s^{-1}—and it maintains exchange over the electrode.) Periodically, we withdrew water samples from the jar and measured the concentrations of ammonia, nitrate, nitrite and phosphate accumulating within the enclosure[15].

The amount of ammonia evolved was quite large (Table 1). Both the oxygen demand of the sediment and ammonia production were highly dependent on temperature. The November experiment came closest to a yearly average temperature and to what we would expect is an average oxygen demand (22.08 ml $m^{-2} h^{-1}$), corresponding to a production of 0.91 mg N (64.6 μg-atom $m^{-2} h^{-1}$), which was close to that actually measured (69 μg-atom $m^{-2} h^{-1}$) evolving into the bell jar. Most of the nitrogen at warmer temperatures was in the form of ammonia. At lower temperatures, some had been oxidised to nitrite and nitrate, suggesting there may have been loss of nitrogen from the system by denitrification to N_2 (ref. 10). On average, 81% of the nitrogen predicted to return to the water did so. Possible sources of error were nutrient uptake by plants in the bell jar, lower-than-normal diffusion out of the mud, or ammonia binding by cation exchange to clay particles[16].

Because nutrients from freshwater could not account for the high near-shore productivity, Riley[1] proposed a model which moved deep nutrients onshore by horizontal advection and tidal mixing. This mechanism is not needed to account for the productivity gradient if benthic feedback (recycling) is included in the system. Bottom respiration on the continental shelf should be near 10–20 ml $m^{-2} h^{-1}$ (refs 11, 12, 14 and 17). This means that 4.12 mg organic carbon and 0.412 mg organic nitrogen (29.4 μg-atom N) would be oxidised to CO_2 or deaminated to NH_4^+, respectively. If about 80% of the NH_4^+ were released from the sediment, the average feedback for the continental shelf would be about 23.5 μg-atom N $m^{-2} h^{-1}$.

We cannot yet confirm this by *in situ* measurements on offshore, continental shelf sediments, though there is strong evidence that our indirect estimates are close to reality (ref. 3, and N. Corwin, unpublished). Ammonia concentrations at continental shelf locations in late summer 1969, south of Long Island (Fig. 1) increased by about 2.5 μg-atom l^{-1} in the bottom 10–15 m of the water column. This gradient was probably maintained by photosynthesis below the thermocline and by vertical eddy diffusion. The flux along the gradient can be represented by a finite difference equation:

$$\text{flux of ammonia} = K\,(\text{change in concentration/change in depth})$$

where K is a 'mixing constant' with values ranging from <1 to >100 (cm^2 s^{-1})[18]. Over a typical sand bottom (Stations 1502, 1503, 1510, Fig. 2) the average near-bottom gradient was about 0.2 μg-atom NH_4^+ l^{-1} m^{-1}. Using 2 cm^2 s^{-1} for K (see ref. 18) we estimate that 144 μg-atom NH_4^+ $m^{-2} h^{-1}$ evolved from the sediment in order to maintain the gradient. The profiles from stations at the New York sewage sludge dump (1504, Fig. 1) and the Hudson Shelf trough had higher bottom gradients. Oxygen demand there at a temperature of 13 °C was 43 ml O_2 $m^{-2} h^{-1}$ (ref. 17), equivalent to a regeneration of 126 μg-atom N $m^{-2} h^{-1}$. The low ammonia concentrations off the continental shelf (Station 1512, Fig. 2) are evidence that the high concentrations of ammonia on the bottom of the shelf did not arise from advection from deep offshore water. Because an intense thermocline existed when these samples were taken,

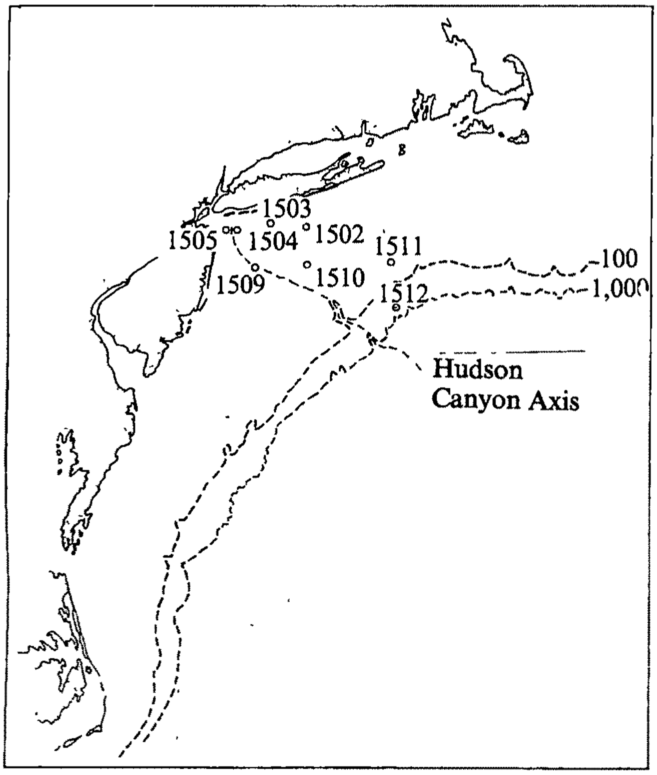
Table 1 Sediment-water nutrient flux

Location and depth	Date	Nutrient flux (µg-atom m^{-2} h^{-1})					Bottom O_2 demand (ml m^{-2} h^{-1})	Predicted N production	Observed N as % of predicted N	Mean O_2 Concentration (ml l^{-1})	Duration (h)	T (°C)
		NH_4^+	NO_2^{2-}	NO_3^{2-}	ΣN	PO_4						
Buzzards Bay (17 m)	November 1973 November 1973	68.8	0.05	0.15	69.00		22.08	64.9	106	4.8	2.15	7.7
Buzzards Bay (17 m)	January 1974	2.56	4.28	4.79	11.63	4.28	10.90	32.1	36	7.8	6.00	1.5
Buzzards Bay (17 m)	February 1974	18.70	1.19	1.19	21.08	5.17	10.35	30.4	69	8.3	2.6	1.6
Buzzards Bay (17 m)	June 1974	124.0	−1.96	2.93	124.97	−14.87	41.89	123.3	101	4.7	5.25	16.0
Eel Pond (2 m)	July 1973	7.87	0.44	1.75	10.06	6.6	14.96	44.0	23	6.2	4.5	20
		117.99	0.94	−1.50	117.43	21.57	51.59	151.8	77	4.7	10.5	20
		127.91	0.46	0.0	128.37	19.46	26.88	79.1	162	2.7	13.0	20
Eel Pond Average		84.59			85.29		31.14	91.6	93			

the ammonia could not have come from Hudson River effluent, because that water would have remained mostly near the surface. The high ammonia content must therefore have come from regeneration on or near the bottom.

We can assess the significance of this recycling in two ways. The area of the New York Bight (about 1.17×10^{10} m^2) would recycle 16.8×10^{11} µg-atom NH_4^+ h^{-1}, if our estimated flux of 144 µg-atom NH_4^+ m^{-2} h^{-1} is correct. On the other hand, the Hudson River and Raritan estuary contribute 8.6×10^{10} µg-atom h^{-1}, if one assumes that the net contribution of freshwater, averaged over one year, is 2.4×10^9 l hr^{-1} (ref. 19), and that the average ammonia concentration for the river water is 35.97 µg-atom NH_4^+ l^{-1} (N. Corwin, unpublished). Another important comparison concerns the nitrogen requirements for photosynthesis. The average primary production rate for Stations 1502, 1503, 1509, 1510 and 1511 (Fig. 2) was 0.150 g C m^{-2} d^{-1} (N. Corwin, unpublished), and if the nitrogen used was about 15% of this, the nitrogen requirement for photosynthesis was 67 µg-atom m^{-2} h^{-1}. Based on our estimate (144 µg-atom m^{-2} h^{-1}), bottom regeneration at the time the data were taken could be supplying more than the total required nitrogen. If there were no contribution from the bottom sediments the system would lose this feedback and the production of plant biomass, dependent only on water column regeneration, would be reduced to rates similar to those found beyond the continental shelf.

Fig. 2 Locations of hydrographic stations shown on Fig. 1. Dashed lines parallel to shore are 100 and 1,000 fathom isobaths.



This work was supported by the National Science Foundation, the International Decade of Ocean Exploration, and the Office of Naval Research.

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Chemical alkylation of lead

FOLLOWING the reported methylation of lead by anaerobic microorganisms[1] we have investigated organolead compounds in aqueous systems. Our results indicate the possible existence of chemical mechanism for the conversion of trimethyl lead acetate, Me_3PbOAc, to tetramethyl lead (TML) in active anaerobic sediments.

Two basic systems were studied. A 'sulphide' system comprising sodium sulphide (hydrated, 15 mg) in distilled water (200 ml) and a 'sediment' system comprising an anaerobic lake sediment (50 ml), a nutrient broth (CM1), (1 g) and glucose (0.2 g) made up to 200 ml with distilled water. All of the systems were sealed by Subaseals in 250 ml volumetric flasks and stored in the dark. The atmosphere above the solutions was sampled by a syringe and tested for tetraalkyl leads by direct injection into a gas liquid chromatography column coupled to an electron capture detector. The identities of the tetraalkyl leads produced were ascertained by comparsion of their retention times with those of authentic samples.

The addition of Me_3PbOAc (15 mg) to a sediment system produced detectable amounts of TML in the atmosphere above the solution after 1 d and the concentration built up gradually to $6 \mu g\ ml^{-1}$ over two weeks. This gives an approximate figure of 300 μg for the total TML in the atmosphere, which compares favourably with the previously reported figures[1] (125 μg after one week and 642 μg after two weeks), considering the unknown amount of TML absorbed in the solution and on to the walls of the vessel.

The addition of Me_3PbOAc (15 mg) to a sulphide system produced TML within 30 min and the concentration built up to $0.5\ \mu g\ ml^{-1}$ in 7 h and $6\ \mu g\ ml^{-1}$ in 2 d. The addition of Me_3PbOAc to distilled water with no added sulphide gave a constant background level of TML of $0.01\ \mu g\ ml^{-1}$. Substitution of trimethyl lead chloride (Me_3PbCl) for Me_3PbOAc gave identical results.

The addition of 15 mg of triethyl lead chloride (Et_3PbCl) to a sediment system produced tetraethyl lead (TEL) in the atmosphere above the solution. This was detectable after 4 d at a concentration of $0.003\ \mu g\ ml^{-1}$. In this time no methyltriethyl lead ($MeEt_3Pb$) was detected. The addition of Et_3PbCl to a sulphide system produced TEL to a concentration of $0.016\ \mu g\ ml^{-1}$ in 4 d. A solution of Et_3PbCl (15 mg) in distilled water (200 ml) produced no detectable TEL in the atmosphere above it.

A sediment system to which no lead had been added produced no detectable tetraalkyl leads. We were also unable to detect any TML from the addition of lead nitrate ($Pb(NO_3)_2$) to sediment systems.

These results suggest that the main reaction producing tetraalkyl lead from trialkyl lead salts in anaerobic systems is the conversion of the trialkyl lead salt to the sulphide, and the subsequent decomposition of the trialkyl lead sulphide $(R_3Pb)_2S$ to form the tetraalkyl lead. The absence of Et_3MePb when Et_3PbCl is added to sediment shows that no direct methylation is occurring in this case.

Sulphide, in the form of hydrogen sulphide is liberated by many organisms under anaerobic conditions. This would normally react with any metallic ions present to form insoluble sulphides (such as, ferrous sulphide) but, presumably, the trialkyl lead salts compete with these inorganic ions for the sulphide. An earlier report[1] stated that no TML was produced after autoclaving of the sediment; in such a system, the amount of free sulphide available will be low because of the reaction of any H_2S present to form insoluble sulphides, and further H_2S will not be made available for the trialkyl lead salt because of lack of biological acitivity. Thus, if the mechanism proposed here is correct, production of TML from Me_3PbOAc would not be expected to proceed after autoclaving of the sediment.

Furthermore, we have shown that one of the major routes in the methylation of mercury in biological systems is not available to these lead compounds. Following the method of Bertilsson and Neujahr[2] for the methylation of inorganic mercury by methylcobalamin, we substituted equimolar quantities of Me_3PbOAc, Me_3PbCl, Me_2PbCl_2 and $Pb(NO_3)_2$ for the inorganic mercury. In none of these cases did methylation occur.

We propose, therefore, that a possible mechanism for the production of TML from Me_3PbOAc in anaerobic systems is the initial formation of $(Me_3Pb)_2S$ followed by decomposition to give TML as one product. This mechanism seems to be dominant in the reaction of Et_3PbCl in anaerobic conditions to form TEL.

We thank the Natural Environment Research Council, and the World Health Organization for support of this work.

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Received March 17, accepted April 11, 1975.

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Accuracy of dates beyond the ^{14}C dating limit using the aspartic acid racemisation reaction

RECENT evidence suggests that the racemisation reaction of aspartic acid can be used to date fossil bones[1–3] (for a general review, see ref. 4). At 20 °C, the half life for aspartic acid racemisation is about 15,000 yr. Because the aspartic acid reaction has a longer half life than radiocarbon, it can be used to date bones which are beyond the ^{14}C dating limit (that is, >40,000 yr old). Also, only a few grams of bone are required for a DL-aspartic acid determination, so bones which are available in insufficient quantities for radiocarbon dating can be dated from the extent of aspartic acid racemisation.

The technique is best applied by 'calibrating' the rate of racemisation reaction, using the extent of racemisation in a bone which has been dated by radiocarbon[1–3]. Once this calibration has been carried out for a particular site, then other bones from the site can be dated based on the extent of their racemisation. Ages deduced using this technique have been shown to be in close agreement with radiocarbon ages[1–3].

It is assumed when using this calibration procedure that the average temperature experienced by the calibration sample is approximately the same as that experienced by the sample which is being dated; a 1° difference in average temperature causes about a 20% change in the racemisation rate constant. Although this equal temperature assumption is certainly valid when the ages of the calibration and dated samples are nearly the same, there is a question of whether it is true when the sample age is much greater than that of the calibration sample. Previous results have suggested that a bone which has a radiocarbon age of about 18,000–20,000 yr is a suitable calibration sample for dating older bones[1–3], particularly those too old for ^{14}C dating. In this paper, we have determined the aspartic acid racemisation ages of several bones which are too old for radiocarbon dating. The aspartic acid ages were deduced using a bone about 17,000 yr old as a calibration sample. The ages derived using racemisation are in very close agreement with the ages deduced from other evidence.

The bone samples used in this study came from the Nelson Bay[5] and Klasies River Mouth (KRM) caves in Southern Cape Province, South Africa (ref. 6 and R. Singer and J. J. Wymer, unpublished). The KRM caves contain a long sequence of Middle Stone Age horizons. Radiocarbon dates[7,8] for various South African sites, including the KRM caves themselves, indicate that the end of the Middle Stone Age lies near or beyond the dating limit of radiocarbon (that is, greater than 35,000 or 40,000 yr).

The extent of racemisation of aspartic acid in the bones was determined using the procedures described elsewhere[1]; in general, about 5–10 g bone was processed. The DL-aspartic acid ratios and their corresponding ages, the stratigraphic

Table 1 Aspartic acid racemisation results for Nelson Bay and KRM caves

Site*	Stratigraphic unit	Depth below deposit surface (m)	DL-aspartic acid	^{14}C age (yr)	Aspartic acid age† (yr)
Nelson Bay cave	YSL‡	1.8	0.151	16,700±240 (I-6516)§	$k_{asp} = 4.92 \times 10^{-6}$ yr^{-1}
	YGL	2.0	0.167	18,100±550 (UW-185)§ 18,660±110 (GrN-5889)¶	20,000
KRM cave I	13	1.5	0.370	> 30,000 yr‖	65,000
	16	1.75	0.467	> 30,000 yr‖	89,000
	18	2.75	0.474	> 30,000 yr‖	90,000
	19	3.0	0.548	> 30,000 yr‖	110,000

*The caves are located in close proximity and have the same general climate, so the calibration value for Nelson Bay cave should be applicable to the KRM cave as well.

†See ref. 1 for equation used to calculate k_{asp} value and the aspartic acid ages.

‡Sample used for site calibration.

§Determined on associated charcoal.

¶Determined on associated ostrich eggshell.

‖The radiocarbon results for these levels are discussed and listed by Klein[8].

relationship of the samples, and the radiocarbon dates are summarised in Table 1.

The geological evaluation of the KRM cave I profile (K. W. Butzer, personal communication), oxygen isotopic analysis of marine mollusc shells from the relevant KRM levels (N. J. Shackleton, personal communication), and Klein's faunal studies[8], strongly suggest that the bulk of the KRM cave I Middle Stone Age sequence was deposited during the last interglacial period. The KRM cave I sequence begins on a raised beach which is 6–8 m above the present sea level (ref. 6 and R. Singer and J. J. Wymer, unpublished). The radiometric dating of fossil corals on Oahu, Hawaii[9], and elsewhere[10–12] has indicated that the last time the level of the sea was appreciably higher than it is today was 120,000 yr b.p. This age must represent the maximum age for the KRM cave I deposit, and is in close agreement with the age of 110,000 yr deduced from the extent of aspartic acid racemisation in bones from the lowest occupational horizon at KRM cave I (that is, unit 19).

The Middle Stone Age sequence at the KRM caves contains marine food refuse including seals, birds, fish and shellfish[8]. This indicates that in the time interval during which Middle Stone Age people occupied the caves, the sea was never very far away. (At present, the cave is 10–15 m from the sea.) Following the high sea level stand of 120,000 yr ago, there was a fairly long interval (about 40,000–60,000 yr) of oscillating sea level[12–16]. Periods when the level of the sea was close to but slightly lower than present levels have been dated[12–15] at about 105,000 and about 85,000 yr b.p.; during the intervening periods sea level was significantly lower than it is today[15,16]. These latter high sea level periods coincide extremely well with the ages deduced for the middle and lower Middle Stone Age occupational horizons (that is, units 16, 18, and 19) at KRM cave I.

Klein[8] states that there is a decrease in the relative proportion of seal and marine bird remains in the youngest Middle Stone Age levels of the KRM caves, including unit 13 of KRM cave I. Following this faunal transition period, the cave was abandoned by Middle Stone Age people and remained uninhabited until about 6,000 yr ago. Klein suggests that this occupational gap coincides with the period of essentially permanent depressed sea level during the height of the last glacial age. Sea level curves given by various workers[13–16] indicate that this period of low sea level began about 60,000–70,000 yr b.p. The aspartic acid racemisation age of 65,000 yr for unit 13 at KRM cave I thus also agrees well with the age range estimated from the faunal and archaeological evidence.

We believe that these results provide convincing evidence that the aspartic acid racemisation can be used to deduce accurate ages of fossil bone which are beyond the dating limit of radiocarbon.

We thank R. Klein for supplying the bone material, for reprints of appropriate papers, and for reviewing the manuscript. This work was supported by a grant from the National Science Foundation.

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Male transfer in olive baboons

STUDIES of human populations have shown that inbreeding results in an increase in the number of offspring with greatly reduced viability. It seems likely that there are similar effects in other primates. In *Papio anubis* it seems that this problem is reduced by the transfer of males between groups. In a population studied at Gombe National Park in western Tanzania, inbreeding may be essentially avoided through the transfer of all males out of their natal troop.

Since 1967, 41 individuals are known to have transferred in or out of three study troops at Gombe. Thirty-nine of these were males, the other two were an adult female and her juvenile daughter. Of the 39 transferring males, two were juveniles at the time of transfer, four were old adults (with worn canines) at their first observed transfer, and the remaining 33 were subadult or mature adults. Of the 18 adult males that currently reside in the three study troops, 13 are known to have transferred in from outside that troop; the other five are all old and could have transferred before research at Gombe began.

In two of the troops, demographic data has been maintained constantly since October 1967, in the third since January 1970. This work was begun by T. Ransom and subsequently continued by L. Taylor-Nash, N. Owens, and A. Sindamwo. Of the 20 oldest subadult males that were members of the study troops when individuals of those troops were first recognised, 15 are known to have transferred, two disappeared, and three died. None have remained in their natal troops. These 15 transfers all left between the ages of approximately 6–8 years; puberty begins at about 5–5½ years and full size is reached at 6½–7½. Although adults which have not yet transferred may attain a high dominance status among mature males, they consistently rank lowest in time spent consorting oestrous females.

Exchange of males at Gombe involves seven different troops. All transfers have been between troops with adjacent ranges. Six males are known to have transferred more than once. Two of these came back to their natal troop, then returned to the transfer troop. The other four are known to have been members of three troops, while one of these is believed to have been a member of four different troops. Three other males are also believed to have transferred more than once, while all the older migrating individuals may have already switched before their initially observed transfer.

Of the males that have reached maturity since 1967, none has remained in his natal troop. Although it is possible for a female to transfer, it is highly unusual. It seems that every male transfers at some time during his life, most likely spending his reproductive life in a troop other than his natal troop. This phenomenon is still being studied in detail.

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Evolutionary trends in the Indian *Iseilema*

OF the most highly evolved grasses, the most useful to man are chiefly confined to the tropical and the warm temperate regions. For example, the Andropogoneae are extensively cultivated for cereals (*Sorghum* spp.), sugar cane (*Saccharum* spp.), essential oils (*Cymbopogon* spp.), and a variety of other purposes. They have attained high adaptive peaks of evolution and species differentiation in the Indo–Malaysian region[1], where there is a high or moderately high rainfall. Three species of the Indian grass *Iseilema* are cultivated for fodder, each one of which is a 'species complex' or a cluster of chromosome races, not randomly united but formed by the genetic system of the taxonomic species. All three complexes show a gradually ascending series of haploid chromosome numbers from 3 to 18 (Table 1), and even the polyploid numbers form an 'aneuploid' series within a complex. Selection, environment, polyploidy and B chromosomes have had varying roles in the evolution of these complexes, and their most notable characteristic is that they form a self-consistent system of evolutionary mechanisms. Here, I demonstrate how *Iseilema* is indicative of the ancient dibasic polyploidy in its tribe, of which it is the highest evolved member; in a habitat where the tribe shows maximum diversity and closest adaptation.

The higher chromosome numbers (Table 1) of *I. laxum* and *I. prostratum* are probably derived from a lower number or numbers by way of remote amphiploidy. For instance, the haploid numbers 14, 14+1B, 15 and 18 of *I. laxum* can all be traced back in a reticulate manner to four secondary basic numbers such as $x_2=6$, 7, 9 and 10 and the latter in turn can be deduced from $n=3$, 4, and 5 of *I. anthephoroides*. The ancient diploid ($n=4$) that is still lingering in the species complex of *I. laxum* lends support to such a view. Hooker[3] stated that *I. anthephoroides* is "nearly allied to *I. laxum*".

Table 1 The ascending series of haploid chromosome numbers of three Indian species of *Iseilema**

Iseilema anthephoroides Hack.	3	Ramana Rao[4]
Iseilema anthephoroides Hack.	3	D'Cruz and Birari[5]
Iseilema anthephoroides Hack.	3+1B	This paper
Iseilema anthephoroides Hack.	4	This paper
Iseilema anthephoroides Hack.	4+1B	This paper
Iseilema anthephoroides Hack.	5	This paper
Iseilema anthephoroides Hack.	5+1B	This paper
Iseilema anthephoroides Hack.	6	This paper
Iseilema anthephoroides Hack.	6+1B	This paper
Iseilema laxum Hack.	4	Celarier and Paliwal[6]
Iseilema laxum Hack.	14	Joshi, Patil and Manchanda[7]
Iseilema laxum Hack.	14	Ramana Rao[4]
Iseilema laxum Hack.	14+1B	This paper
Iseilema laxum Hack.	15	This paper
Iseilema laxum Hack.	18	Ramanathan[8]
Iseilema prostratum (Linn.) Anderss.	10	This paper
Iseilema prostratum (Linn.) Anderss.	14	Joshi, Patil and Manchanda[7]

* Pending a careful biosystematic study to validate the taxonomic characters such as sterility and variation in the relative lengths of the spikelets[16], sometimes caused respectively by B chromosomes and polygenes, Hooker's species[3] are still maintained.

In spite of many cycles of polyploidy (the highest known to date in the genus), some plants of *I. laxum* ($n=14$) have one B chromosome, and seem to have been selected and retained within the complex, whereas others, with $n=15$ and 18, do not. The B chromosome may play a role in restoring vigour, presumably lost as a result of functional diploidy in these lower polyploids; such a source of vigour, as alternative to polyploidy, is no longer required when the basic number increases and hence the higher polyploids tend to eliminate B chromosomes in *I. laxum*.

I. anthephoroides, however, shows an imposing array of as many as eight different chromosome numbers with one B chromosome at each stage, the presence or absence of which does not affect the chiasma frequency at metaphase I in pollen mother cells. Here, selection is progressing not in favour of an increase but towards constancy in the number of B chromosomes, resulting in balanced polymorphism. In this continuous series of chromosome numbers there is at the moment no way of predicting which, if any, is the most primitive. This difficulty is further enhanced by the fact that several basic numbers have been proposed for the Andropogoneae[9]. If statistics alone can provide an unequivocal answer, then $x=5$, the most frequent number, is an important evolutionary radiation in the Andropogoneae and the same set of five chromosomes is also basic for *Iseilema*. At one time, the Andropogoneae were probably in a state of great flux, centred round a basic set of five chromosomes, from which not only several genera like *Iseilema* but even the highest evolved Maydeae seem to have originated.

Themeda, considered to be phylogenetically related to *Iseilema* (Hubbard and Bor, personal communication), is also based on $x=5$ (refs. 10 and 11). *Themeda* and *Iseilema* may have had a common ancestor with five basic chromosomes. Further, the supposed $x=5$ of *Iseilema* need not be derived from $n=10$, encountered in *I. prostratum* and in other Andropogoneae, through such numbers as $x=9$ and $x=7$ also found in *I. laxum* and *I. prostratum*, because $x=9$ and $x=7$ are in themselves amphiploid and can easily be derived from the $n=3$, 4 and 5 of *I. anthephoroides*; similarly, with $n=6$ which co-exists with the $n=3$.

If $x=n=5$ is accepted as a working hypothesis, the haploid number $n=3$ in *I. anthephoroides* can be derived directly or indirectly from $x=5$ by a progressive and stepwise reduction in the basic number (for example, *Airopsis tenella*[12]), also providing desirable centromere economy. Such a chromosome reduction seems to have gone on unobtrusively in such tribes as Aveneae, Stipeae and Agrosteae in parallel and independent lines but not to the extreme extent found in the Andropogoneae. Parallel chromosome reduction in these grass tribes may be indicative of their genetical relationship and common inheritance from the ancestral grass stock, which has emerged[13] from the Liliaceous stock through Juncales by the process of extreme reduction. Thus, instead of being merely a basic number[5], $n=3$ of *I. anthephoroides* represents a highly advanced number, perhaps the culmination in the tropical Andropogoneae.

What is remarkable in *I.anthephoroides* is not the stringent centromere economy as a measure of achieving the long range advantage in evolution, but the acquisition of B chromosomes to compensate for a decrease in the number of normal chromosomes. It may well be that a reduction to such a low number as 3, the lowest known to date in grasses, has become an evolutionary possibility resulting from the material properties of B chromosomes, which probably belong to an ancient lineage. In other words, the B chromosomes seem to act as buffers to the haploid chromosomes and provide a mechanism for an elastic adjustment of the increased nucleic acid metabolism, as demanded by the evolutionary changes, both within and outside the species.

It is clear that *I.anthephoroides* has achieved internal stability by an extreme reduction in the haploid number to 3 and plasticity by still retaining excessive variability in the haploid number from 3 to 6, with one B chromosome at each stage. These are two conflicting but delicately balanced evolutionary needs which seem to be advantageous to the species. It is in fact a compromise imposed by selection.

What do we learn when the chromosomal evolution in *Iseilema* is related to its geographic distribution? With all the advantages that a balanced polymorphism can confer on a species population, *I.anthephoroides* is still confined to the southern Deccan Peninsula. This may be caused by the fact that, along with increasing specialisation, the species has become adapted to a particular set of environmental conditions obtained only in that area; or the B chromosomes have adaptive properties of their own restricting the species to its present range of distribution (for example, *Clarkia lingulata*)[14]. In contrast, the species complex of *I.laxum* has combined polyploidy and one B chromosome with adaptability, although its area is not commensurate with the high degree of its ploidy, indicating that at least some of its chromosome sets are probably not qualitatively very different from each other. It is distributed over the entire Deccan Peninsula and in Ceylon with a peripheral distribution of its sole diploid ($n=4$) in the Assam area. The geographic distribution of *I.prostratum*, is far wider, covering all parts of India and extending into Burma. Its success cannot be attributed to polyploidy alone, which is not even as high as that of *I.laxum*, but presumably to the onset of a favourable recombination through hybridisation of genetic types, adapted to diverse habitat conditions, such as those in the black cotton soils of Bundelkhand and the moist localities of the Carnatic. Relatively, therefore, it is not the step-wise reduction, but the origin of secondary basic numbers through amphiploidy, that has decided the areas of the Indian species of *Iseilema*. Their ultimate adaptation to specialised habitat conditions within the areas result from intraspecific genetic variation within the complex.

The Andropogoneae have attained a high development in the Indo–Malaysian region, with two zones of maximum species abundance, one in India (the Western Ghats, Bombay Deccan, Madhya Pradesh) and the other in Southern Indonesia (the Lesser Sunda Islands)[1]. It is in this Indian zone of strong species preponderance, that *Iseilema* shows maximum cytological variability with unusually low chromosome numbers, providing a 'pool of chromosome sets', from which the higher chromosome numbers have been derived mostly by amphiploidy and to some extent by autopolyploidy.

Such amphiploidy, involving any two parents with $n=3$, 4 or 5 and leading to the origin of secondary, tertiary and other complex basic numbers, explains the wide gamut of seemingly conflicting basic numbers, such as 5, 6, 7, 9, 10, 11, 12, 14, 15, 17, 18 and 19 encountered in the tribe Andropogoneae of the Indo–Malaysian region[9]. Perhaps it is the differential elimination of certain unsuitable types with $x=8$, 13 and 16 that has created gaps in this otherwise continuous series. It seems that all the basic numbers 3, 4 and 5 were present in the primitive flux of the Andropogoneae making the dibasic polyploidy and the evolution possible in the moist habitats of the Indo–Malaysian region, Hartley's ideas[1] being vindicated. The cytology of the Indian *Iseilema* thus puts the whole tribe Andropogoneae in its proper perspective both in regard to the long range chromosome reduction and the short range polyploidy, and takes us to the very root of Poaceae which, according to some cytologists, is characterised by a basic set of five chromosomes. In other words, the cytology of *Iseilema* illustrates the great series of chromosome changes which the tribe Andropogoneae has undergone since its early divergence from the original grass stock.

I thank Dr C. E. Hubbard, Royal Botanic Gardens, UK, and Professor Arne Müntzing, Institute of Genetics, Lund, Sweden for their interest.

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Received December 20, 1974; accepted March 19, 1975.

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Genetic relationships between brooding and brooded *Actinia tenebrosa*

SEA anemones of the genus *Actinia* seem to be dioecious and reproduce sexually, yet males, females, and individuals without gonads brood juveniles for much of the year[1–3]. How this occurs is still not understood and knowledge on the subject is scant. Gillespie[4] considered that eggs develop to ciliated blastulae and then directly into "a cylindrical form . . . which develops tentacular buds". Chia and Rostron[3] found no pre-planula larval stages in the coelenterons of hundreds of specimens they examined and postulated that most embryos must leave the mother as morulae or blastulae and later resettle in the coelenteric cavities of other adult *A. equina* where they metamorphose to post-Edwardsia juveniles. According to this hypothesis, juveniles contained in an adult should be derived from a number of different parents and would be expected to show a range of phenotypes characteristic of the population rather than the brooding adult. Cain[5], however, found that in phenotypically varied populations of *A. equina*, young anemones invariably had the same colour as the adult from which they were released. He considered it likely that *A. equina* is a protandric hermaphrodite which mainly

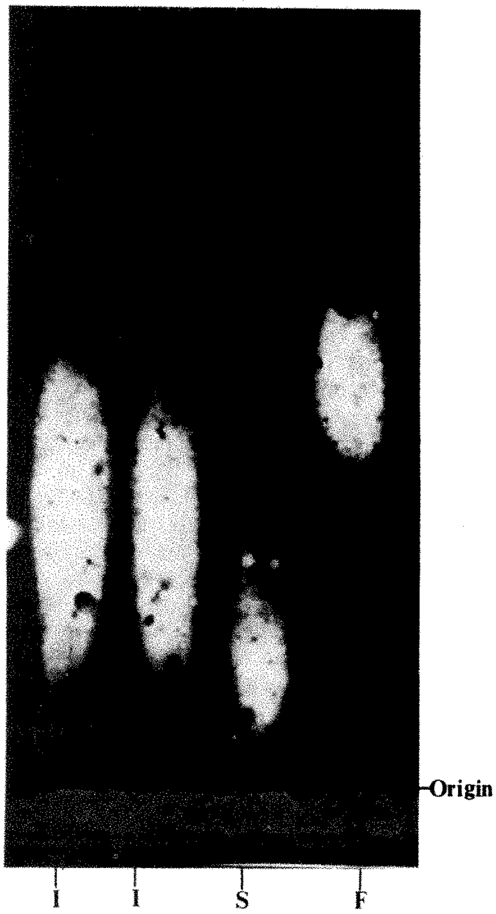

Fig. 1 Starch gel electrophoresis of *A. tenebrosa* catalase in 0.045 M Tris, 0.0008 M Na_2 EDTA, 0.025 M H_3BO_3, *p*H 8.6 buffer. Catalase stain modified from Shaw and Prasad[8]. I, intermediate phenotype; F, fast pheonotype; S, slow phenotype. Samples were frozen, ground in an equal volume of distilled water and centrifuged at 2,000*g*.

self-fertilises and retains larvae within the parent, even though Carlgren[2] thought this "hardly probable".

The intertidal anemone *A. tenebrosa* Farquhar is common on the shores of New Zealand and temperate regions of Australia. There is only one recorded colour phenotype[6], so we examined *A. tenebrosa* for isozyme polymorphisms, similar to those known in *A. equina*[7], to test Chia and Rostron's[3] hypothesis.

Homogenised sea anemone tissue extracts were examined for eight enzymes[8] using starch and gradient acrylamide ('Gradipore') electrophoresis. No traces of peroxidase or leucine aminopeptidase activity were found; there was no individual variation in soluble protein, esterase, acid phosphatase, alkaline phosphatase, alanyl aminopeptidase, or malate dehydrogenase. Three catalase phenotypes were found; fast, intermediate and slow (Fig. 1). From earlier studies of catalase isozyme polymorphisms[9,10] we assumed that *Actinia* catalase is a tetrameric molecule; thus the fast and slow phenotypes were assumed to be homozygotes, whereas the intermediate phenotype was a heterozygote of 'fast' and 'slow' alleles. The intermediate phenotype of *Actinia* catalase should therefore contain five isozyme bands, but these were not distinguishable on our gels.

The sea anemones were collected from four localities near Adelaide, South Australia (Table 1). Phenotype frequencies varied between localities and in the Outer Harbour sample there was a highly significant excess of putative heterozygotes, assuming a diploid diallelic system, with respect to Hardy–Weinberg frequencies. Some large brooded juveniles from the Outer Harbour sample provided sufficient catalase to give a staining reaction after electrophoresis. Phenotypes of 23 juveniles were determined (Table 2). Every juvenile examined had the same catalase phenotype as the host adult.

Table 1 Catalase phenotype frequencies from different localities

Samples	Phenotypes			X test for Hardy–Weinberg frequencies	
	Slow	Intermediate	Fast		
Outer Harbour (Adelaide)	6	59	2	39.46	$P<1\%$
Victor Harbour	0	3	9	—	—
Talisker Mines Beach (Cape Jervis)	0	5	5	—	—
Pennington Bay (Kangaroo Island)	16	5	0	0.38	—

Our results are therefore consistent with those of Cain[5], but we feel that the information available is insufficient and too contradictory to allow detailed speculation on the nature of the breeding system in *Actinia*. The correlation between phenotypes of adults and their brooded juveniles could indicate either asexual reproduction or apomictic parthenogenesis, but these systems would be inconsistent with previous observations of apparently functional male and female gonads. Furthermore, recent work raises an unlikely possibility that has not been dismissed: that a brooding adult may differentiate genotypes of brooded juveniles and reject those incompatible with its own.

Table 2 Phenotypes of juveniles extracted from adult *A. tenebrosa* in the Outer Harbour sample

Catalase phenotype of juveniles	Catalase phenotypes of nurse adults		
	Slow	Intermediate	Fast
Slow	5	0	0
Intermediate	0	15	0
Fast	0	0	3

Francis[11] showed that *Anthopleura elegantissima* distinguished between contacts of clonemates (of like genotype) and non-clonemates (of unlike genotypes), and reacted aggressively to the latter. *Actinia* also exhibits intraspecific conflicts[12], which may result in death of a juvenile involved in an adult–juvenile encounter (J. R. O., unpublished). Successfully brooded juveniles could therefore be only those genetically similar to the host, regardless of whether they resulted from cross-fertilisation of one or many matings. One approach to resolve this problem would be to breed adult *Actinia* in captivity for several years and compare fecundity of animals in solitary and group isolation with similar individuals and groups that were not isolated but had access to planulae from the sea.

Finally, we comment on Cain's assumption that *A. equina* is "so long lived". Substantial longevity data are available for only four species of sea anemones, all of which are for captive animals[13–16]. Only two of these records are for *A. equina*: one of Dalyell's specimens lived about 66 yr[14] and another lived 14 yr[13], but the lifespan of animals in such conditions may far exceed "ecological longevity" since a captive animal is usually protected from predators, hazards of the environment and scarcity of food[17]. Thus, although some actinians may have the potential to live many decades, it is possibly quite a rare occurrence in nature.

This study was supported by Dr F. Jacka, Director, Mawson Institute for Antarctic Research, and by a

University of Adelaide Research Grant Scholarship to G. C. K. during 1971–72.

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Received February 24; accepted March 17, 1975.

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Epidermal mucus and the reproduction of a crinoid echinoderm

THE unstalked crinoid *Comanthus japonica* spawns during the first part of October each year[1,2]. For a study of gametogenesis[2], specimens of *Comanthus* were collected periodically over 14 months. During these collections, one of us (J. G.) noticed that the animals became unusually slimy to the touch during the six to eight weeks that preceded spawning. This transient slimyness is correlated with an enlargement of an epidermal mucous cell population. The general position of the mucous cells is shown in Fig. 1, in which they are darkly stained. In both male and female genital pinnules, these cells occur mostly in the oral and lateral body wall. Each individual mucus cell is oval and contains a single nucleus. The cytoplasm stains with beta- to gamma-metachromasia in azure A. In addition, the cytoplasm stains strongly with alcian blue at *p*H 3, but fails to stain with periodic acid–Schiff. These histochemical criteria indicate an abundance of sulphated acid mucopolysaccharides (possibly mixed with non-sulphated acid mucopolysaccharides). It is not known if similar mucous cells occur in the arms and central mass of the crinoids, since these body regions were not preserved for histology.

In both sexes, the volume of the epidermal mucous cell population increases conspicuously in the weeks before spawning (Fig. 2). In the female (Fig. 2*a*), this increase is about ten times as great as in the male (Fig. 2*b*). The volume of the mucous cell population decreases markedly around the time of spawning in October in the female, but not in the male. Instead, the volume in the male declines gradually during the autumn and winter.

In both males and females, the population volume of the mucous cells increases abruptly in August. Therefore, the increase might be elicited in both sexes by the same environmental signal. The much greater increase in females could result from endocrinological differences between males and females; or there might simply be more mucous cells (or their precursors) in females. At present, it is not known if mucous cells are derived from or give rise to other cell types in adult crinoids. There is certainly no morphological evidence that the mucous cells are related to the so-called dorsal glands[4] of the body wall.

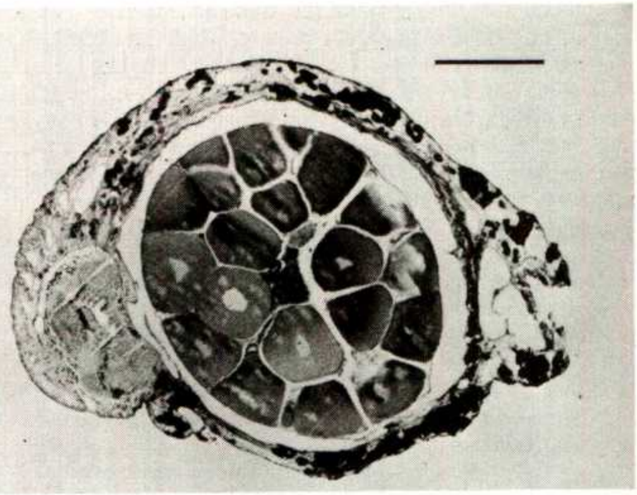
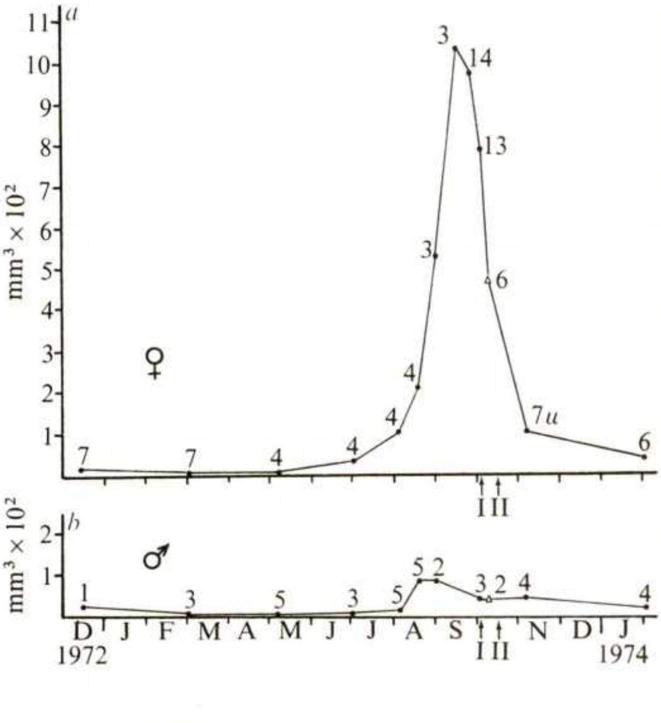


Fig. 1 Cross section of a genital pinnule from a female specimen of *Comanthus japonica* collected on September 15, 1973. The oral side with the food groove is at the right and the aboral side with the pinnular ossicle is at the left. The ovary, containing conspicuous oocytes, is separated from extragonadal portions of the pinnule by a genital coelom. Fixation was on the day of collection in seawater–Bouin's fluid. Staining[3] was in alcian blue at *p*H 3. The epidermal mucous cells are the darkly stained objects in the oral and lateral body wall. The scale line represents 250 μm.

The marked fluctuations in the size of the mucous cell populations around the time of spawning indicate that the epidermal mucus has some function in the reproductive biology of female crinoids. It is possible that the release of much epidermal mucus into the seawater by spawning females may act as a pheromone to stimulate epidemic spawning in nearby crinoids. Dan and Dan[5] have shown, however, that such a pheromone is not necessary in all cases, since a male crinoid can spawn in isolation from spawning females (and *vice versa*).

Fig. 2 Volume fluctuations of epidermal mucous cell populations in females (*a*) and males (*b*) of *Comanthus japonica*. Volumes, given in $mm^3 \times 10^2$ per genital pinnule, were measured by the method of Holland *et al.*[2]. The number of the individual females or males averaged for each data point is given above each collection date. For the collection of October 7, 1973, (△), only spawned animals were taken into account; the spawnings of the first and third weeks of October 1973 are marked, respectively, by arrows I and II. The November data point marked 'u' on the top graph is for animals which could not be sexed, most or all of which were probably females (see Holland *et al.*[2]).



Another, and more probable, function of the female's epidermal mucus may be to supply a temporary binding material for the newly released eggs. Dan and Dan[5] discovered that the eggs of *Comanthus* are mixed with a viscous binding material which rises slowly through the seawater in a column above the spawning female. It was assumed[5] that this material was produced within the ovary from dissolution of the jelly coats of eggs. But much (perhaps all) of the binding material may be supplied by the epidermal mucous cells. If there is a sudden secretion of a large part of the epidermal mucus at spawning, the epidermal mucous cells may well be responding to the same hormone(s) that presumably direct maturation, ovulation and spawning in this species.

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Siphon regeneration in *Ciona*

IN 1923 Paul Kammerer summarised research which he claimed demonstrated the inheritance of acquired characteristics. Since the success of Mendelian genetics had already made Kammerer's work suspect, a report of his lecture[1] was followed by a long and acrimonious debate. The Kammerer case thereby became a *cause célèbre* in the biology of that decade. The controversy has recently been resurrected in a popular book by Koestler[2] and has gained additional prominence from an internationally televised BBC documentary based on the book. This paper reports a failure to duplicate one of Kammerer's key experiments.

One of the examples cited by Kammerer purported to show that environmentally induced nuptial pads on the forelimbs of the male midwife toad, *Alytes obstetricans*, were inherited by the male offspring[3]. In 1926, however, this work was discredited by the finding that the nuptial pads on his one remaining *Alytes* specimen in the museum of the Vienna Institute for Experimental Biology were fraudulent[4]. Although Kammerer protested innocence of the forgery, the disgrace contributed to his suicide shortly afterwards[5].

Koestler has staunchly defended Kammerer's scientific integrity[2], and on the basis of his presumed innocence in the matter of the midwife toad, and on the grounds that Kammerer's experiments were never properly repeated, he urges the scientific community to reinvestigate the work. Although there is ample reason to disagree with Kammerer's (and Koestler's) Lamarckian interpretations[6], some of his experimental observations may nevertheless still be valid.

Kammerer himself believed that his experiments on siphon regeneration in the ascidian *Ciona intestinalis* L. were his best evidence for the inheritance of induced somatic changes[1,7,8], and Koestler particularly seeks a repetition of this work[2]. The original experiment and its results are summarised in Fig. 1. Removal of parts of the incurrent and excurrent siphons (Fig. 1*a*) resulted in more elongated siphons being regenerated, even after a single excision. After a second removal of the newly regenerated siphons, still longer siphons were produced (Fig. 1*b*). The gonads were then made to regenerate by removing a basal segment of the animal containing the ovary and lower part of the intestine (Fig. 1*b* and *c*); Kammerer felt that the regenerating gonads would be more amenable to somatic influences. The F_1 progeny of these gonad-regenerated animals were shown to have elongated siphons (Fig. 1*d*) like the parents.

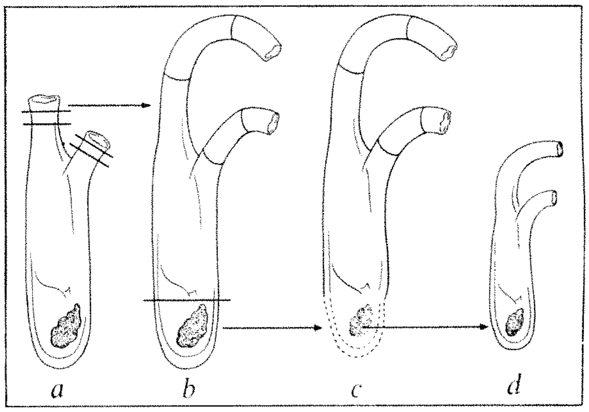

Fig. 1 Diagrammatic representation of the *Ciona* regeneration experiment purportedly done by Kammerer[8]. *a*, Distal halves of the incurrent and excurrent siphons of the animals were excised (cuts indicated by the solid line) and permitted to regenerate twice in succession, to produce animals with exceptionally long siphons as depicted in *b*. Regenerated siphons had a jointed or noded appearance. *b*, Gonad of the long-siphoned *Ciona* made to regenerate by cutting off (solid line) the basal part of the body which contains the gonad. *c*, Long-siphoned animals which had regenerated new basal parts and gonads (shown by dotted lines) were used to provide eggs and sperm for an F_1 generation. *d*, Young *Ciona* (F_1) resulting from this genetic cross were reported to have long siphons like the parents.

In response to Koestler's appeal[2], I attempted to repeat this regeneration experiment during the summer of 1973. Adult *Ciona intestinalis* (8–12 cm long) were kept at Woods Hole in a tank in which the incoming fresh seawater was refrigerated to 12–15 °C. Cionas survive much better in cooler water than in the warmer laboratory sea tables. Before surgical amputations, the animals were anaesthetised with 0.01% tricaine methanesulphonate (MS 222; Sandoz Pharmaceuticals).

Two groups of 50 animals each had both siphons amputated: group 1 had the whole siphon removed at the base, and group 2 had the distal half of each siphon removed. Healing was quite rapid[9] and new ocelli (reddish-orange photoreceptors[10]) appeared on the siphon edges within 4–6 d. Animals of both groups had completely regenerated their siphon lengths by 30–40 d, and showed no indication of siphon elongation nor any sign of a jointed or noded appearance of the siphons[8].

A control group of 50 unoperated animals was kept in the same tank for comparison of relative siphon lengths. Group 1 were kept up to 60 d (38% survived compared with 68% of the control group) without any indication of excessive siphon growth. Likewise, the group 2 animals were kept for 53 d (56% surviving), also without siphon elongations beyond their normal proportions. Since siphon elongation was reported to occur even after a single amputation[8], it seemed pointless to amputate the animals a second time.

These results agree with those of Fox[11], who attempted to repeat the *Ciona* experiments at the time of the original controversy. In addition to Kammerer's report, however, an earlier worker had also described siphon elongations in *Ciona* after a single amputation of the siphons[12]. A suggestion[11] that these positive results could be caused by nutritional conditions which were known to produce elongated siphons in *Ciona*[13], was flatly rejected by Kammerer[14].

No attention seems to have been paid during the controversy[2] to the final phase of Kammerer's experiment in which he claimed to have obtained gonad regeneration. Gonad regeneration occurs in another ascidian, *Clavellina lepadiformis*[15], and recent work shows that *Ciona* will regenerate a new ovary if the original is carefully removed through a small slit in the body wall[16]. Anyone actually trying the final operation depicted in Fig. 1*b* and *c*, however, could not fail to be impressed by the improbability of the animals surviving such trauma. In fact, Hirschler[17], in his extensive study of *Ciona* regeneration, found that animals could not survive this particular operation.

This point was investigated further by subjecting a third group of 50 normal animals to the gonad operation described by Kammerer[8], and as depicted in Fig. 1*b*. All of the animals died between 3 and 6 d after the operation.

The failures of Fox[11] and myself to observe siphon elongation in regenerating *Ciona* suggest that elongation is not a commonplace occurrence and may indeed be caused only by special circumstances[13]. Kammerer's claims must therefore continue to be regarded with scepticism, especially as evidence presented here and elsewhere[17] indicates that *Ciona* does not survive the gonad regeneration operation claimed to have been used by him.

This investigation was supported by a US Public Health Service research grant.

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Received December 30, 1974; accepted April 2, 1975.

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Temperature receptors on tarsi of the tsetse fly *Glossina morsitans* West

ELECTROPHYSIOLOGICAL techniques have shown that the responses of mechano- chemo- and olfactory receptors in insects are temperature-dependent[1-3], and several true thermoreceptors have been described[4-8]. We have found thermosensitive cells on the tarsi of the tsetse fly that seem to be involved in probing behaviour. Electrophysiological recordings revealed spontaneously discharging cells in two hairs on the third, fourth and fifth prothoracic tarsomeres. These hairs are part of two ventrolateral rows of hairs probably similar to the receptors (re) described by Lewis for *Glossina palpalis*[9]. No spontaneously firing cells were found on the meso- or metathoracic legs. Detailed studies were made only on the fifth tarsomere hairs.

Table 1 Probing responses of *G. morsitans*

	n	No. of flies settled on membrane	No. of flies probing	No. probing / No. settled
Post-teneral males				
Accumulated controls	96	90	84	0.93
Antennae removed	43	?	2	
Pro. tarsi removed	61	57	47	0.82
Acetic acid on meso. tarsi	9	8	7	0.79
Acetic acid on pro. tarsi	33	26	3	0.12
Teneral males and females				
Accumulated controls	60	56	54	0.96
Pro. tarsi removed	45	41	26	0.63
Acetic acid on meso. tarsi	32	27	25	0.93
Acetic acid on Pro. tarsi	29	26	6	0.24

Post-teneral males were 1–3 weeks old and had been starved for 48 h. Teneral flies were starved for 24–48 h. Cages containing five to seven flies each were placed for 3 min on a 1-mm thick Agar/Parafilm membrane[13] overlaying a feeding solution (10^{-3} M ATP in 0.15 M NaCl, warmed to 37 °C with *p*H adjusted to 7.0). Glacial acetic acid was applied after the inconclusive results of the tarsalectomy experiments. Electrophysiological experiments had shown that application of the acid to the tip of the hair gives an injury reaction: a burst of spikes followed by inactivity of the temperature receptor. To apply the glacial acetic acid, a glass tube (outer diameter about 1 mm), filled with the chemical, was placed over the leg of the fly. In this way evaporation of the acid, which may cause damage to other receptors, was minimised.

At room temperature, there were usually two cells firing in each hair. One gave large spikes (0.8–1.0 mV) at irregular intervals (on average 1–2 s^{-1}) and the other gave smaller spikes (0.3–0.6 mV) at a frequency of 20–40 s^{-1}. The large spikes gave occasional bursts, which often, though not always, coincided with a change in temperature. Air movement alone did not evoke the bursts, and some occurred without any detectable stimulus. Over a temperature range of 16–32 °C, the cell giving the small spikes showed all the characteristics of a cold receptor as defined by Hensel[10] (Fig. 1). At other temperatures the amplitude of the spikes usually decreased to zero. Below 18 °C the decrease in amplitude was sometimes preceded by a sudden drop in frequency.

A sudden change in temperature caused a phasic increase

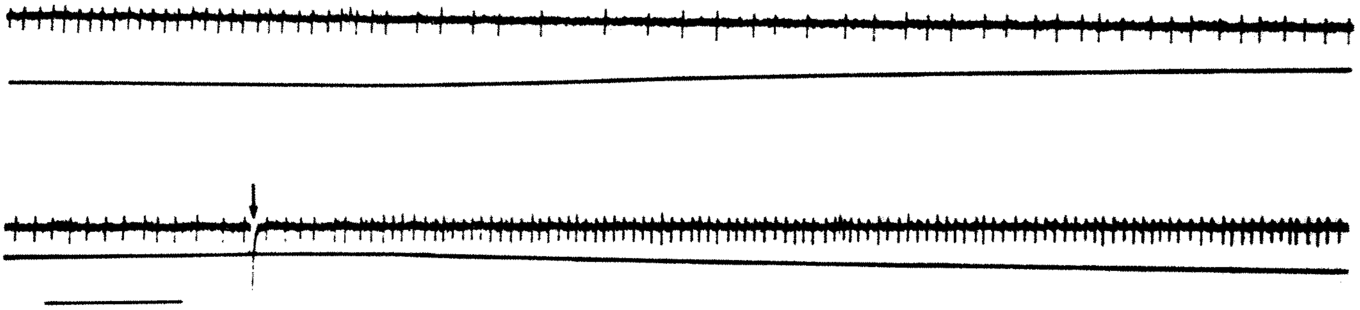

Fig. 1 Record of responses from a hair on the fifth tarsomere containing a cold receptor. The time bar represents 0.25s. The fly's leg was cut off at the base of the tibia and mounted on a glass pipette filled with saline (0.15 M NaCl) which served as the indifferent electrode. Extracellular recordings were made from the tip of the hair by placing a glass pipette (outer diameter of tip 6 μm) containing saline over the hair[11]. A silver wire in the saline passed the signal to a W-P, VF-1 voltage follower, and the output was viewed on a Tektronix oscilloscope. Records were made with a Honeywell 1858 oscillographic recorder or with a Grass C-4 camera. Air at 2–50 °C was blown from a glass condenser over the leg, to obtain an ambient temperature range of 15–35 °C measured with a small (diameter 0.095 inch) epoxy coated thermistor mounted approximately 5 mm from the tip of the hair. The top trace represents the response of the cold receptor to an application of a warm airstream, while the third trace represents the response of the same cell to removal of the warm airstream (arrow and electrical artefact). The recording is continuous. The line under each electrophysiological trace represents the response of the thermistor. The response of the thermistor is not necessarily a true indication of the rate or magnitude of the temperature change at the surface of the receptor. With longer applications than shown here, however, it was possible to get an approximation of the magnitude of the temperature change when the thermistor had stabilised (see Fig. 2*a*).

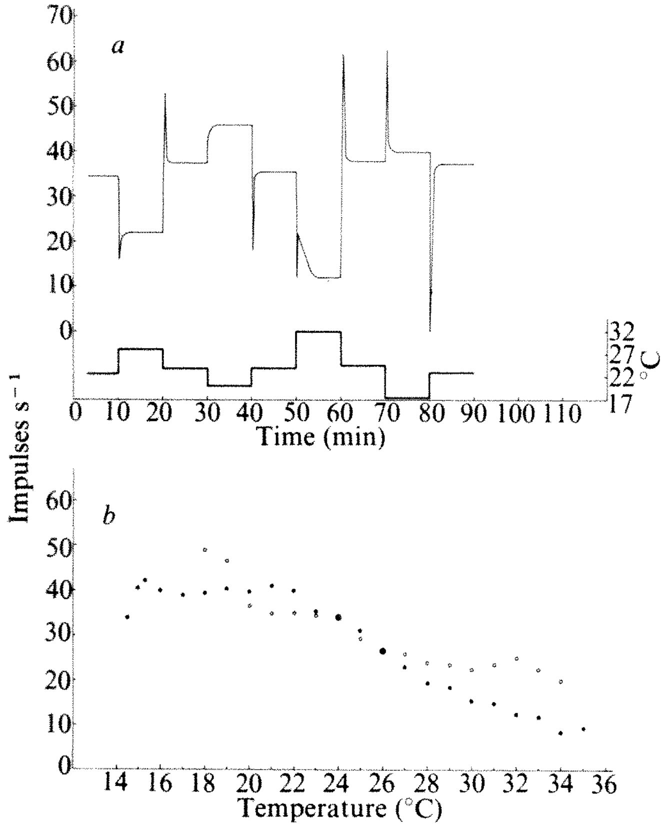

Fig. 2 *a*, Responses of a single cold receptor (top graph) to rapid changes in temperature followed by steady temperatures (bottom graph). The air in the condensor was held at various temperatures (above or below room temperature) and then applied suddenly to the preparation (see Fig. 1). The preparation was held subsequently at each new temperature for 10 min before being returned suddenly to room temperature for 10 min. An approximation of the magnitude of the temperature change was obtained in each case when the thermistor equilibrated. The changes are plotted as instantaneous, and must have been very fast, at the receptor level, judging from its response. *b*, Response of a single cold receptor to slow changes in temperature. The temperature was allowed to increase from about 15 °C to over 34 °C, and the response of the cell was monitored at regular intervals (●). After 169 min, the temperature was decreased, and cell response was again plotted (○) for 64 min.

(cooling) or decrease (warming) of spike frequency, of a size determined both by the extent of the temperature change and by the initial steady temperature (Fig. 2*a*). Frequencies up to 80 spikes per s were recorded in response to a rapid decrease in temperature. When temperature changed slowly (1 °C per 3–5 min), from approximately 21 to 34°C, the firing frequency of the cell followed almost linearly (Q_{10}=0.4–0.7) (Fig. 2*b*). Above 40 spikes per s tonic reactions of the cell became unpredictable.

To test the possibility that the temperature receptor may also respond to chemicals, NaCl (1.0 M), urea (0.1 M) and LiCl (0.3 M) were each applied to the hair. In each case, the temperature receptor functioned normally.

Of what value are these thermosensitive cells to the tsetse fly? Electrophysiological identification of such receptors gives no clue to any influence they may have on the animal's behaviour. Dethier[12] showed that heat is the major stimulus that induces probing, and that the receptors detecting the heat source are on the antennae, rather than on the palpi. Although he mentioned temperature sensitivity of the legs, only chemical responses were studied in detail. When we removed the prothoracic legs, results were variable but there tended to be a reduction in the number of flies which probed. This was easier to demonstrate in teneral flies (newly emerged flies before their first meal) than in post-teneral flies. Treatment of the prothoracic legs with glacial acetic acid eliminated the probing response in most teneral and post-teneral flies (Table 1). In a control group, with mesothoracic legs treated with glacial acetic acid, the probing response was not altered significantly. We conclude that, in its probing response, the tsetse fly uses a secondary input from the temperature receptors on the tarsi, in addition to the primary input from the receptors on the antennae. Why acetic acid treatment disrupted probing more effectively than removal of the prothoracic legs is unknown.

We thank Dr R. H. Gooding for helpful discussions, and for tsetse flies and Dr E. E. Davis of Stanford Research Institute, Menlo Park, California for his unpublished manuscript. This work was supported by the US Army, Medical Research and Development Command, and was carried out while H. R. v H. held a Canada Council Scholarship.

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Received December 23, 1974; revised March 28, 1975.

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Passive immunisation of marmoset monkeys against neoplasia induced by a herpesvirus

THE malignant lymphoma induced by herpesvirus saimiri (HVS) in non-human primates[1] provides a model system for the study of the prevention of neoplasia in man induced by suspected oncogenic herpesviruses[2]. We have reported that cotton topped (CT) marmoset monkeys (*Saguinus oedipus*) actively immunised with a killed HVS vaccine were resistant to the inoculation of the oncogenic HVS while the non-vaccinated control monkeys died of malignant lymphoma[3]. In certain virus infections (for example, measles, infectious hepatitis, German measles) passive immunisation by administration of specific serum antibodies during the incubation period may result in prevention or modification of the clinical disease. This paper describes the prevention of herpesvirus induced malignant lymphoma in monkeys by passive immunisation.

For all experiments described HVS isolate S.295C[1] was used. The virus was cultivated on owl monkey kidney (OMK) cells[4] and stored at 4 °C as stock virus. For the passive immunisation of CT marmosets whole serum passed through Millipore membrane filters (450 nm) was used. The monkeys received the immune serum by intramuscular inoculation in the lower extremities and were challenged 24 h later by intramuscular inoculation of HVS in an upper extremity. Serial tenfold dilutions of filtered (450 nm) stock virus were prepared for challenge. Aliquots (1 ml) of each dilution were injected into passively immunised monkeys and also into non-immunised control monkeys. The infectivity titre ($TCID_{50}$) of the virus dilutions was determined in parallel in OMK cells.

CT marmosets with HVS induced malignant lymphoma developed high titres of specific serum antibodies against HVS[5]. In the first experiment immune serum obtained from tumour-bearing CT marmosets was used. One of the two immune sera tested was obtained from a CT marmoset that had received 2×10^6 freshly prepared tumour cells derived from an animal of the same species with HVS induced

Table 1 Challenge of CT marmoset monkeys with live HVS

	Dilution of HVS stock virus	Titre of inoculum		Survival time after inoculation of HVS (d)	Infectivity titre of whole blood (TCID$_{50}$ ml^{-1})‡
		LD$_{50}$*	TCID$_{50}$†		
Passively immunised monkeys	10^{-1}	3,162	1,000	134§	$10^{5.0}$
	10^{-3}	32	10	73§	$10^{5.5}$
	10^{-3}	32	10	79§	$10^{5.5}$
	10^{-3}	32	10	346¶	$< 10^{0.0}$
Non-immunised control monkeys	10^{-1}	3,162	1,000	33§	$10^{5.3}$
	10^{-3}	32	10	23§	$10^{2.5}$
	10^{-3}	32	10	45§	$10^{6.5}$
	10^{-3}	32	10	47§	$10^{5.5}$

The monkeys were passively immunised with serum obtained from CT marmosets which had died of malignant lymphoma.
*Determined in experiment No. 2 (Table 2).
†Determined in OMK cells.
‡Determined in OMK cells at the given survival time.
§Monkey died of malignant lymphoma.
¶Monkey is clinically well.

malignant lymphoma. The recipient CT marmoset died of malignant lymphoma 36 d after the cell transplantation. The serum was taken from this monkey at the time of death. The neutralisation index of this serum determined in OMK cells according to standard techniques[6] was 3.0. The serum (6 ml) was inoculated in a CT marmoset. This monkey was challenged with the 10^{-1} dilution of the stock virus. The TCID$_{50}$ of the 10^{-1} dilution was 1,000 (Table 1) and corresponded to 3,162 LD$_{50}$ calculated according to the method of Reed and Muench (Table 2). The immunised monkey showed delayed tumour development and survived 134 d whereas the non-immunised control monkey died 33 d after inoculation of malignant lymphoma (Table 1). The second immune serum was obtained from four CT marmosets that had been inoculated with 10 TCID$_{50}$ of cell free HVS stock virus. All four monkeys had died of malignant lymphoma 34–42 d after infection. The neutralisation index of the pooled serum from these monkeys was 2.8. Three CT marmosets each received 6 ml from this serum pool. These three monkeys were challenged with the 10^{-3} dilution of the stock virus which contained 10 TCID$_{50}$ of HVS corresponding to 32 LD$_{50}$. While the three non-immunised control monkeys died of malignant lymphoma 23–45 d after inoculation, two of the passively immunised monkeys died of the tumour as late as 73 and 79 d after inoculation and the third of the passively immunised monkeys survived without any sign of a clinical disease or infection with HVS as monitored by cocultivation of its peripheral blood with OMK cells[7]. This animal has now been under observation for 346 d (Table 1).

For the second experiment the serum for the passive immunisation was obtained from CT marmosets actively immunised with a killed HVS vaccine as described previously[8]. None of these monkeys showed signs of a HVS infection[4]. Blood was drawn from 24 vaccinated CT marmosets at different time intervals after the immunisation with the HVS vaccine and the sera were pooled. The neutralisation index of the serum pool was 3.5 and the titre of the complement fixing (CF) antibodies was 1 : 32. The CF titres were determined according to standard techniques[6] using a HVS antiserum from an owl monkey[4]. Six CT marmosets each received 6 ml of this serum. The titres of neutralising serum antibodies in the peripheral blood of these monkeys were determined 24 h later, before challenge. At this time the neutralisation indices ranged from 1.8 to 2.0. After 103 d they had dropped to nearly zero. These monkeys were challenged with the 10^{-1} and 10^{-3} dilutions of the stock virus. Both the passively immunised monkeys that had been

Table 2 Challenge of CT marmoset monkeys with 10 and 1,000 TCID$_{50}$ of live HVS

	Dilution of HVS stock virus	Titre of inoculum		Survival time after inoculation of HVS (d)	Infectivity titre of whole blood (TCID$_{50}$ ml^{-1})†
		LD$_{50}$	TCID$_{50}$*		
Passively immunised monkeys	10^{-1}	3,162	1,000	63‡	$10^{5.5}$
	10^{-1}	3,162	1,000	74‡	$10^{5.5}$
	10^{-3}	32	10	204§	$< 10^{0.0}$
	10^{-3}	32	10	204§	$< 10^{0.0}$
	10^{-3}	32	10	204§	$< 10^{0.0}$
	10^{-3}	32	10	204§	$< 10^{0.0}$
Non-immunised control monkeys	10^{-1}	$10^{3.5}$	$10^{3.0}$	31‡	$10^{4.8}$
	10^{-1}	$10^{3.5}$	$10^{3.0}$	34†	$10^{5.0}$
	10^{-3}	32	10	42†	$10^{5.5}$
	10^{-3}	32	10	44‡	$10^{5.5}$
	10^{-3}	32	10	45‡	$10^{5.5}$
	10^{-3}	32	10	52‡	$10^{5.5}$
	10^{-4}	3.2	1	36‡	$10^{4.8}$
	10^{-4}	3.2	1	44‡	$10^{5.5}$
	10^{-4}	3.2	1	52‡	$10^{5.3}$
	10^{-4}	3.2	1	126‡	$10^{4.5}$
	10^{-5}	< 1	< 1	204§	$< 10^{0.0}$
	10^{-5}	< 1	< 1	204§	$< 10^{0.0}$
	10^{-5}	< 1	< 1	204§	$< 10^{0.0}$
	10^{-5}	< 1	< 1	204§	$< 10^{0.0}$

The monkeys were passively immunised with serum obtained from CT marmosets actively immunised with a killed HVS vaccine.
*Determined in OMK cells.
†Determined in OMK cells at the give observation time.
‡Monkey died of malignant lymphoma.
§Monkey is clinically well.

challenged with the 10^{-1} dilution died of malignant lymphoma. The tumours appeared only after a delay, however, and the monkeys died 63 and 74 d after the challenge whereas the non-immunised control monkeys died 28 and 34 d after inoculation (Table 2). All of the four passively immunised monkeys that received the 10^{-3} dilution survived without any sign of disease or virus replication and have now been under observation for 204 d. In contrast, all of the non-immunised monkeys that received the 10^{-3} dilution died 23–47 d after inoculation of malignant lymphoma and even all four of those control monkeys that received the 10^{-4} dilution developed a tumour (Table 2).

These experiments demonstrate clearly that non-human primates can be protected against HVS induced malignant lymphoma by passive immunisation. The passively immunised CT marmoset monkeys were resistant to small dosages of cell-free HVS during an observation period of 204 and 346 d whereas the non-immunised control monkeys died of malignant lymphoma. Challenge with doses higher than 32 LD_{50} of HVS regularly induced a tumour in the passively immunised CT marmosets. The development of malignant lymphoma, however, was considerably delayed in these monkeys as compared with the non-immunised control monkeys. The serum obtained from tumour-bearing CT marmosets protected another CT marmoset against a tumour of the same kind. The fact that the humoral antibodies were not able to stop the growth of an established tumour in the donor of the serum indicates that a passive immunisation may only protect if carried out before the intracellular establishment of the infection. Humoral antibodies do not protect against the cellular transmission of herpesviruses[9] and antibodies against herpes simplex virus types 1 and 2 provide little protection against recurrent disease in man[10]. The active immunisation of CT marmosets with a killed HVS vaccine[8] protected against malignant lymphoma induced by the inoculation of cell free HVS[3]. The fact that the protection could be transmitted with the serum of the vaccinated monkeys indicates that humoral antibodies are important in resistance to HVS in those experiments. In addition, the results obtained with passive immunisation against HVS support the concept that HVS is the aetiological agent for the tumours.

We thank Mr M. Bolay for discussions and Mrs G. Spiegel and Mrs A. Moawad for technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

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Differential heat response of normal and transformed human cells in tissue culture

MUCH interest has been rekindled in the possible use of hyperthermia either alone or as an adjunct to more conventional treatments in the management of malignant disease. There are several reasons for this renewed interest: data indicate that malignant murine cells may be more sensitive to increased temperature than their normal counterparts[1,2]; temperatures in the range 39–43 °C sensitise cells, and particularly radioresistant hypoxic cells, to X rays[3–6] and to chemotherapeutic agents[7–9]; and humans can stand prolonged exposures to temperatures up to 43 °C, provided adequate supportive precautions are taken[10,11].

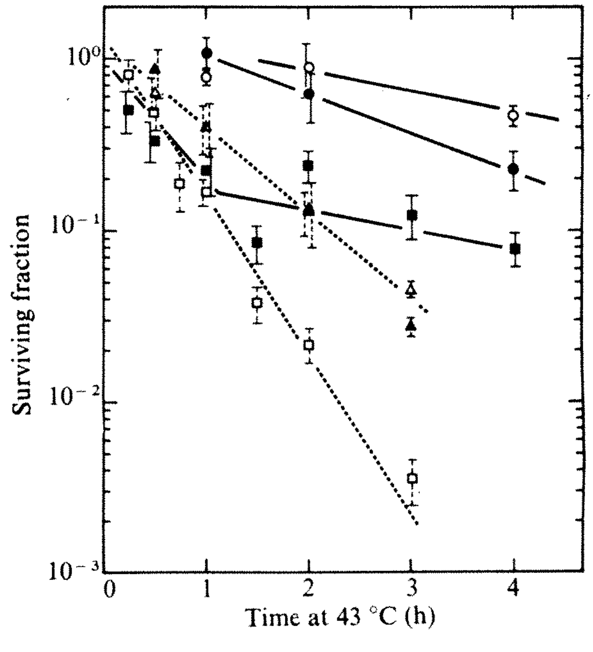
Surprisingly, the literature contains essentially no quantitative data on the thermal response of normal human cells; *a fortiori* there are no data on comparative response of normal cells and similar cells shown to be malignant by implantation into immunodeficient animal hosts. We have therefore initiated such an investigation using the embryonic lung fibroblasts WI-38[12] and SV-40 transformed WI-38-VA13/2RA[13]. Our findings indicate that, indeed, the transformed cells are consistently more sensitive to 43 °C exposures. Furthermore, we show that the kinetics after heat shock of the two cell populations are different, a finding which may have application in sequential heat–X ray or heat–chemotherapy.

Both cell strains were tested for tumorgenicity by injecting 2×10^5–2×10^6 cells subcutaneously into the flanks of homozygotic *nu/nu* mice developed on an NIH background at Stanford Medical Center and maintained under aseptic conditions. One of five mice injected with WI-38-VA13/2RA cells developed a visible nodule within 10 d of injection. None of the three mice injected with WI-38 cells developed nodules. These results are similar to those published elsewhere[14] for other malignant human cells. Furthermore, essentially similar results were shown after implantation of cells of a similar origin as WI-38 and WI-38-VA13 cells into terminal cancer patients[15,16].

The cells were plated in 60 mm plastic Petri dishes at 1×10^5–2×10^5 cells per dish in 5 ml of Eagle's MEM supplemented with 15% foetal calf serum medium. Cells in exponential growth were used on either day 1 or day 2 after plating. The population doubling time in exponential growth varied from 22 to 32 h. Cells reached a confluent monolayer in 9 to 11 d after plating with no change of medium. These plateau-phase cells were used either with no change of medium or with fresh medium applied on the day before heating and again on the day of heating.

Cells were heated by placing replicate dishes in a rack which

Fig. 1 Survival of exponentially growing cells (■, WI-38 and □, 2RA) and plateau-phase cells (●, WI-38, fed; ○, WI-38, unfed; ▲, fed; △, 2RA, unfed) exposed for various times at 43 °C. Unfed cells had no medium change since planting. Fed cells had a medium exchange on the day before and the day of the heat treatment. Error bars represent 1 s.e.m.



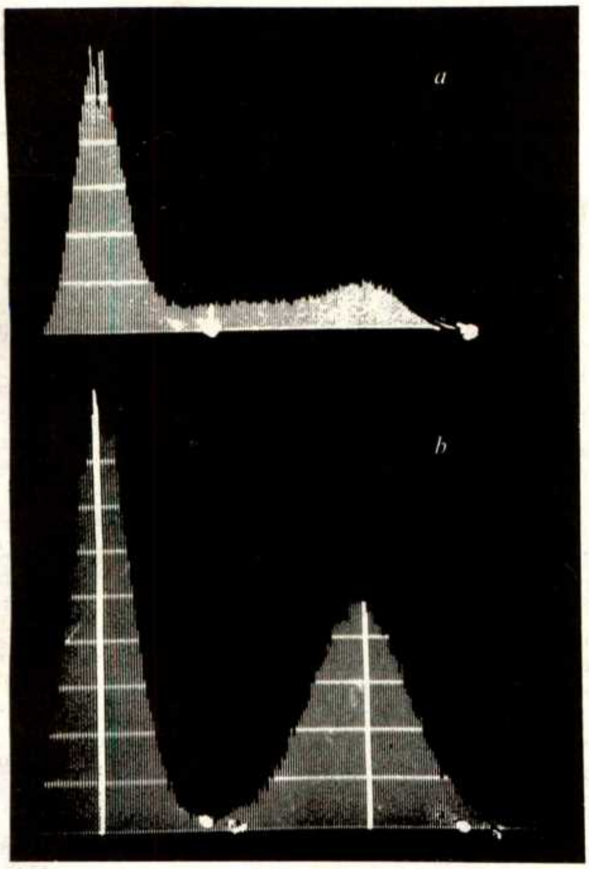


Fig. 2 DNA distributions of WI-38 cells. *a*, Normal distribution of exponentially growing cells; *b*, distribution of cells 24 h after heating at 43 °C for 4 h.

allowed the cells and growth medium to be immersed in a 43 °C water bath. The water bath was inside an incubator and a mixture of air and 5% CO_2 was bubbled through the water constantly. Water temperature was controlled to ±0.1 °C by a proportional controller (Versatherm Model 2156, Cole-Parmer Co.).

For survival experiments, cells were detached after heat treatment using 0.05% trypsin and replanted at the appropriate dilution in 60-mm or 100-mm plastic Petri dishes for cloning at 37 °C. Cloning efficiencies varied from 5% to 35%. Survival results, illustrated in Fig. 1, show that generally the transformed cells exhibited a lower survival than the normal cells when given the same exposure to 43 °C in the same conditions of cell growth, confirming observations with murine cells[1,2]. Fresh medium seems to have had a slight sensitising effect on the normal cells. Also the exponentially growing cells survived less well than plateau-phase cells. This result is contrary to that found with one other mammalian cell line[17].

To determine the effect on cell population kinetics during and after exposure to 43 °C, we studied the changes in the DNA distribution of the cell populations after heating. Several dishes of exponentially growing cells were heated at 43 °C for a fixed time. At various times up to 6 d after heat treatment, replicate dishes were trypsinised and the cells were fixed and stained with ethidium bromide[17]. The cell suspension was then passed through a commercially available fluorescence flow system (Cytofluorograph 4801, BioPhysics Systems, Inc.); output signals from which were accumulated on a multichannel analyser. Since the uptake of dye per cell is directly proportional to DNA content[18], the analyser output is a representation of the DNA distribution of the cell population. Typical distributions are shown in Figs 2 and 3, which also show typical shifts in the distributions after heating. Cells in G1 appear in the first peak while cells in G2-M, with a double DNA complement, appear in the second peak. S-phase cells fall in between the two peaks.

A sample containing equal numbers of each cell strain was also analysed to determine the relative DNA complements of the two strains. This analysis shows that the DNA complement of the transformed cells was almost twice that of the normal diploid cells, confirming that tetraploid cells dominate the population in the transformed strain[13].

Changes in the DNA distributions with time after heating at 43 °C are shown in Fig. 4. The points represent data from two or three experiments. The heating times were chosen to give about the same fractional survival in both cell populations (0.05). Accumulation of S-phase cells while the cells were at 43 °C together with a depletion of cells in G2-M may indicate a heat-caused block in the progress from S to G_2 in the transformed cells. Depletion of G1 also indicates a mitotic block during heating. After the return to 37 °C there was a steady increase in the G2-M population in both cell strains for 20–60 h, with a corresponding decrease in G1 and S populations. During the first 20 h this accumulation was probably the result of a continuing mitotic block for all cells. The populations were dominated by non-viable cells during the first few days after heating; the continuing accumulation in G2-M is thought to result from an accumulation of cells in late G2 or mitosis before cell death. Beyond 60 h viable cells have proliferated sufficiently to influence the DNA distributions and these gradually return to normal.

Assuming that the WI-38-VA13/2RA cells model the malignant cells of a human tumour whereas the WI-38 cells model the associated normal tissue, there seem to be inherent

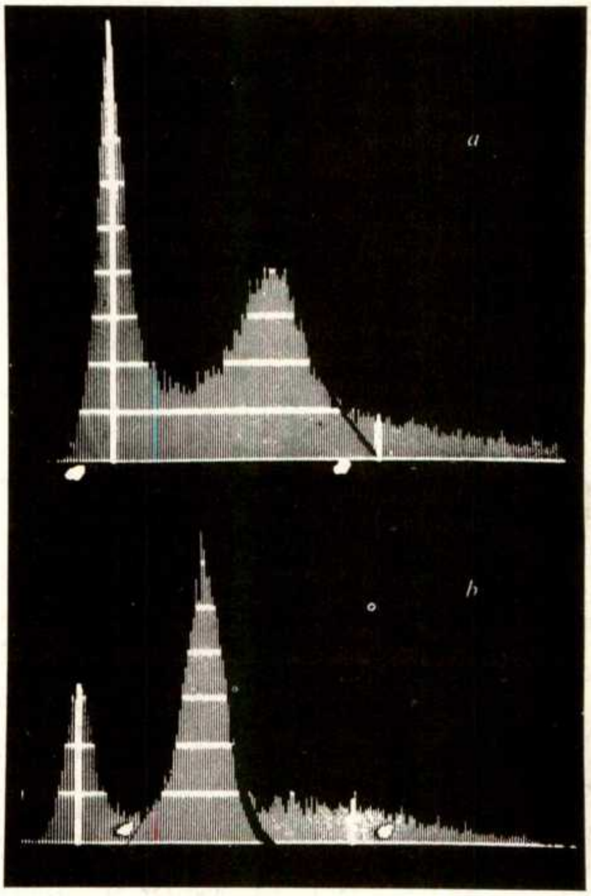


Fig. 3 DNA distributions of WI-38-VA13/2RA cells. *a*, Normal distribution of exponentially growing cells; *b*, distribution of cells 46 h after heating at 43 °C for 90 min.

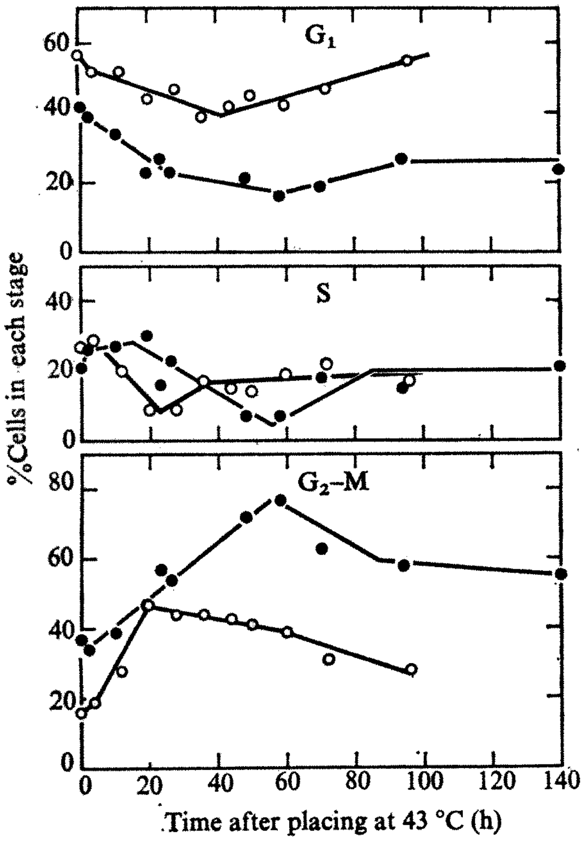


Fig. 4 Variation with time after heating of the DNA distribution for WI-38 cells heated at 43° C for 4 h (○) and WI-38-VA13/2RA cells heated at 43 °C for 90 min (●).

differences in the hyperthermic response which may be exploited in cancer treatment. Not only are the transformed cells more readily destroyed by hyperthermia, but the differential cell kinetics that follow heating suggest that fractionated treatments involving either hyperthermia alone or in combination with radiotherapy or chemotherapy could further improve the therapeutic ratio.

We thank Dr L. Hayflick for WI-38 cells and Dr A. J. Girardi for WI-38-VA13/2RA cells. We also thank Drs H. Kaplan and A. Epstein for supplying the nude mice and for assisting in the tumorgenicity test, and Mr M. Hatfield for obtaining and analysing the DNA distributions. This work was supported by a US Public Health Service grant.

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Received February 21; revised March 31, 1975.

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Organ specificity of blood-borne tumour metastasis determined by cell adhesion?

ONE of the most clinically troublesome aspects of cancer is metastasis, the spread of tumour cells from primary to distant, multiple secondary sites[1–3]. Metastasis initially occurs through tissue invasion, followed by transportation of tumour cells through the circulatory system, lymphatics or coelomic cavities[1,2]. In blood-borne metastasis free tumour cells or cell aggregates are carried into various organs and tissues where they stop in the first capillary bed encountered; however, most of these arrested tumour cells break loose, recirculate and stop in other organs[4,5]. In many tumour systems the location and extent of established secondary tumour colonies are non-random, suggesting that unique cell properties are important in tumour arrest and metastasis[3,6]. In support of this hypothesis Fidler[7] was able to select from a murine B16 melanoma line variants that showed enhanced metastatic behaviour by repeated cycling of tumour cells from lung colonies to tissue culture, administering the tumour cells intravenously on each cycle. We report here the organ adhesive specificity of four B16 variant cell lines selected for enhanced lung metastasis.

B16 variant melanoma lines with increasing ability to form lung metastases from subcutaneous or intravenous administration were obtained from Dr I. J. Fidler (University of Pennsylvania). These B16 lines are designated F1, F5, F10 and F13 based on the number of *in vivo* selections. The metastatic properties of these B16 variants are stable and not dependent on cell growth conditions[7]. Melanoma variants were grown *in vitro* in Dulbecco's modified Eagle's MEM[8] (DMEM) supplemented with 10% foetal calf serum, glutamine and non-essential amino acids. Cells were collected with EDTA as described previously[9]. The variant lines were also grown *in vivo* by intraperitoneal implantation in syngeneic C57BL/6J mice. After 2 weeks cells were collected from the peritoneal cavities and gently dispersed in DMEM through 200-mesh stainless steel filters. Contaminating phagocytic cells averaged less than 5% and viability assessed by dye exclusion averaged greater than 96%.

To assay organ cell adhesive properties single cell suspensions of lung, liver, kidney, brain and spleen were obtained from naive, syngeneic animals as follows. Organs were removed from 6–12 adult mice, pooled according to organ type and cut into 2–4-mm pieces in 10 ml of cold DMEM without serum and washed in DMEM. All steps were performed at 4° C. Dispersion of organs into single cells was accomplished by gently teasing the tissue pieces over the stainless steel filter with a Teflon policeman. Cell suspensions were rinsed by centrifugation (800*g*, 10 min) twice with 15 ml volumes of 0.05 M sodium phosphate-buffered saline (PBS) *p*H 7.4. Single, viable organ cells were isolated from cell debris, erythrocytes, platelets and cell aggregates by the two-step isopycnic-rate zonal gradient technique of Perper *et al.*[10]. Isopycnic centrifugation in Ficoll-Hypaque gradient yielded viable single cells with some membrane contamination. Subsequent centrifugation in discontinuous sucrose density gradients (5–20–30% w/v sucrose in PBS) separated erythrocytes, platelets and membrane debris (5% layer) and some minor cell-aggregates (30% layer) from single organ cells (20% layer). Final organ cell suspensions were rinsed free of sucrose by centrifugation in PBS and contained less than 5% erythrocytes by conventional

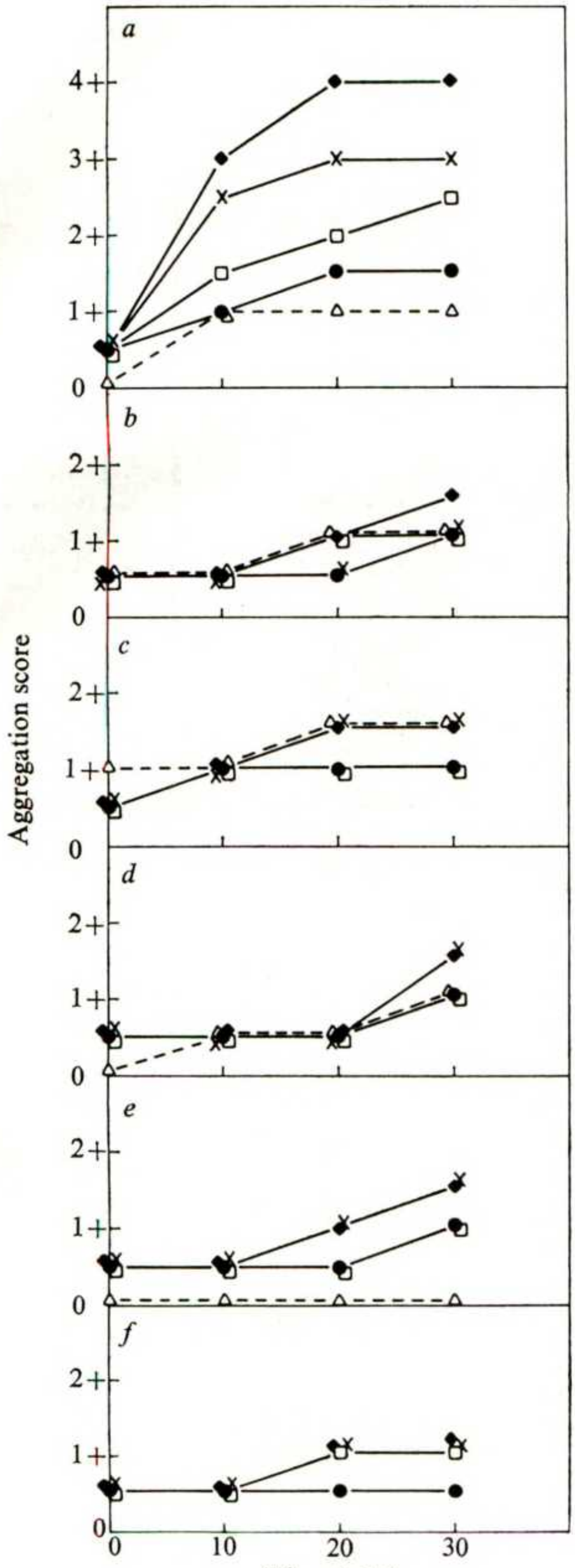

Fig. 1 B16 tumour cell aggregations with murine C57BL/6J organ cells. *a* Lung; *b* liver; *c* spleen; *d* kidney organ cell suspensions; *e* erythrocytes and *f* tumour cells alone. Extent of aggregation is scored from 0 (no aggregation) to 4+ (complete aggregation) (see text for details on the assay). These results were confirmed in three separate experiments using coded triplicate samples for each point. B16 melanoma cells were used at 1×10^6 ml^{-1}. ●, F1; □, F5; ×, F10; ◆, F13 and △, organ cells. Lung, liver, spleen and kidney were used at 3×10^6–5×10^6 ml^{-1} whereas the erythrocyte concentration was 1×10^7 ml^{-1}.

Wright's stain. Viability was greater than 97% as judged by dye exclusion, and contamination by phagocytic cells averaged less than 7%.

The aggregation assay was performed in 0.4 ml of medium containing 2% foetal calf serum using Linbro FP54 trays. Cells were dispensed into each well on the basis of their approximate surface area (see legend to Fig. 1), and the trays were rotated at 2 Hz on a rotary platform[9,11,12] for various times at 37 °C. Aggregation was scored as before[9] from 0 (no aggregation; 90–100% single cells), 1+ (scant aggregation; 60–90% single cells), 2+ (moderate aggregation; 30–60% single cells), 3+ (strong aggregation; 5–30% single cells) to 4+ (complete aggregation; 0–5% single cells). The reliability of this assay has been monitored and confirmed by electronic particle counting techniques[12] and adherence of ^{125}I-5-iodo-2′-deoxyuridine-labelled cells to cell monolayers J. L. W. and G. L. N., unpublished).

When melanoma variant cell lines are suspended with partially purified organ cells, heterotypic aggregation occurs[13]. This aggregation is specific and is related to the preferred organ site for implantation. The tumour lines selected for more lung metastases showed greater organ specific aggregation: lung>liver, kidney, spleen, erythrocytes (Fig. 1). With suspended lung cells the extent of aggregation paralleled the number of metastatic selections that the lines had undergone *in vivo:* F13>F10>F5>F1 (Fig. 1*a*). There was less aggregation with non-target organ cells, with little indication of selective aggregation by the more metastatic lines (Figs 1*b*–*e*). B16 tumour cells also homotypically (self) aggregate (J. L. W. and G. L. N., to be published). There was significantly less homotypic than heterotypic aggregation with lung cells (Fig. 1*f*), but the

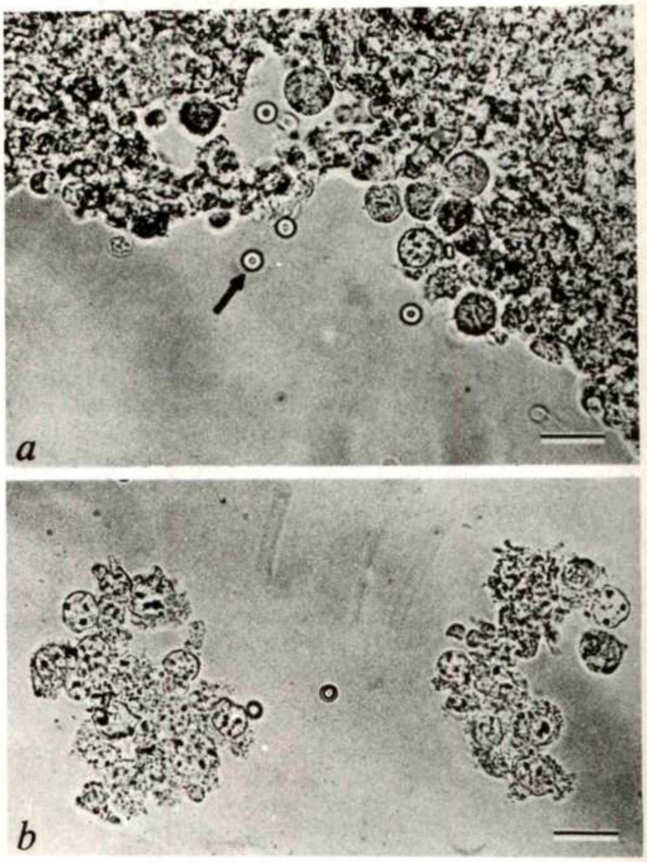

Fig. 2 Heterotypic cell aggregates formed with B16 variants and organ cells. *a*, B16-F13 variant aggregated with lung cell suspension for 20 min at 37 °C results in complete aggregation and the formation of one large mixed cell aggregate (4+ aggregation); *b*, B16-F5 variant aggregated with lung cells for 20 min at 37 °C (2+ aggregation). Arrow indicates unaggregated erythrocyte. ×420; bar represents 10μm.

difference was small or non-existent in homotypic and non-lung heterotypic aggregation experiments (Fig. 1*b–e* and our unpublished results). Microscopic examination of wells containing 4+ aggregates of lung and F13 cells revealed practically no free cells, indicating complete aggregation (Fig. 2*a*). Heterotypic aggregates formed with lung cells and B16 lines or with other organ cell suspensions were heterogeneous in cell composition (Fig. 2*b*). The existence of mixed aggregates of tumour and organ cells was confirmed by double labelling techniques (work unpublished). Soon after initiating the assay (10 min) there was little evidence of nonspecific cell aggregation (Fig. 1*e–f*).

The preferential adhesion to lung cells of the increasingly metastatic B16 variant lines seems to be paralleled by tumour cell behaviour *in vivo*. Injection of ^{125}I-labelled cells of the B16 variant into the tail vein of syngeneic mice results in 100% lung arrest of the B16-F11 and -F6 lines and approximately 80% lung arrest of the F1 line within 2 min. By 4 h 90, 80 and 55% and after 3 d 47, 35 and 2% of lines F11, F6 and F1, respectively remain in the lungs[14]. These results parallel closely the number of resulting lung tumour colonies visible within 3–4 weeks in the animals. Subcutaneous implantation of B16 metastatic variant cells in syngeneic hosts followed by removal of the primary subcutaneous tumours 1 to 2 weeks later results in spontaneous lung metastases within 6–8 weeks, the number formed being proportional to the number of *in vivo* selections F10>F5>F1 (ref. 14). These results indicate that initial cell–cell recognition during tumour cell arrest in specific organs may be one of the most important steps in the spread of blood-borne tumours.

Recent observations suggest that the success of tumour implantation and subsequent metastatic growth are determined by unique cell surface properties. Treatment of tumour cell surfaces with trypsin, neuraminidase[15–17], heparin[18,19] or dextran[20] before intravenous introduction modifies arrest and tumour distribution. Surface interaction of blood-borne tumour cells with lymphocytes[14] and platelets[21] can also affect the fate of circulating tumour emboli. Using B16 metastatic variants, Bosmann *et al.*[22] reported differences in surface proteases, glycosidases, glycosyltransferases and electrophoretic mobilities between low and high metastatic lines. These studies suggest the importance of cell surface properties in determining the *in vivo* fate of tumour cells.

Our results argue against an entirely nonspecific trapping mechanism for circulatory tumour cell arrest[1,2] and suggest that cellular recognition is important in determining the organ specificity of metastatic tumour spread. Since highly metastatic B16 tumour cells cause virtually complete aggregation of suspended lung cells, it is possible that recognition occurs through surface structures common to most, if not all, lung cells. Several types of cells are obtained by the dispersion techniques used here (for example, lung tissue yields endothelial, type I and II epithelial cells, fibroblasts and others) (Fig. 2), but contaminating blood cells such as erythrocytes, platelets, glass-adherent phagocytic cells and so on can be removed by density gradient separation, particle phagocytosis and other techniques. The role of blood cell interactions in the arrest of tumour emboli[14,21] was not examined here, but we are investigating the adhesion of tumour cells to platelets, lymphocytes and macrophages that affect implantation. The ultimate biochemical determination of the surface structures involved in the adhesion of tumour cells to organ cells is feasible using closely related B16 tumour lines with different recognition and metastatic properties.

These studies were supported by a contract from the Tumour Immunology Program of the US National Cancer Institute and grants from the US Public Health Service, Human Cell Biology Program of the US National Science Foundation and the Jane Coffin Childs Memorial Fund for Medical Research. J. L. W. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research at the Salk Institute.

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Received February 18; revised April 2, 1975.

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Embryonic use of egg shell calcium in a gastropod

THE function of the calcified outer eggshell found in many gastropod eggs which are deposited on land has been the subject of considerable speculation. Various theories of its relationship to desiccation, mechanical support, protection against predators, physiological buffering and possible calcium source have been discussed[1-4]. This report presents the first quantitative analysis proving that a gastropod embryo uses the egg shell calcium in forming its embryonic shell, the protoconch.

The egg of the land pulmonate *Anguispira alternata* (Stylommatophora: Endodontidae) was examined during its development. This snail deposits eggs which have a heavily calcified, solid $CaCO_3$ shell made of calcite[5]. Each snail deposits 20–50 eggs, 2.5 mm in diameter, at 20 min intervals during a laying period. Hatching time is approximately 50–60 d at 20 °C; in land pulmonates there is usually a very large amount of variance in the rate of development and consequently in the time of hatching.

The egg shell and egg contents (albumen fluid) were analysed separately for calcium. After 20, 30, and 43 d incubation at 20 °C the eggs were opened and the embryos and egg shells analysed separately for total calcium content (Tables 1 and 2). In the freshly deposited egg, the albumen and single-celled

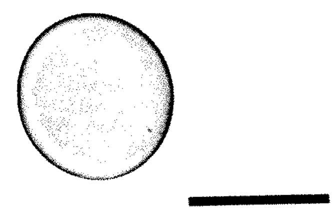

Fig. 1 Soft X-ray radiograph of a live, newly deposited *Anguispira alternata* egg. Bar represents 2 mm. For the generation of soft X rays, a GE Special Model F-5 machine with a beryllium tube and tungsten target was used. Operating conditions were 25 kV at 5 mA with Du Pont Ortho-Cronar Litho Film.

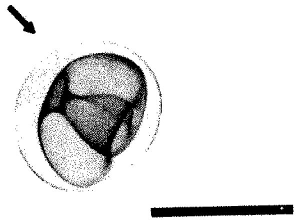

Fig. 2 Egg incubated at 20 °C for 43 d. The egg shell is now thinner and the embryonic shell, the protoconch, has formed at its expense. Arrow indicates where the snail will hatch. Live egg, exposure and printing as for Fig. 1. Bar represents 2 mm.

zygote together contain less than 1% of the total egg calcium; all the rest is in the egg shell. Between 20 and 30 d incubation, the embryo begins to absorb significant amounts of calcium from the egg shell. By 43 d of development, the embryos have absorbed up to 60% of the egg shell calcium. The range of results in Table 2 is a reflection of the staggering of developmental rates in land snails.

Table 1 Mean calcium distribution in the freshly deposited egg

Sample	Sample size	mg Ca per egg
Intact egg (shell + albumen)	5	0.608
Intact egg	5	0.490
Albumen	10	0.003
Albumen	5	0.002

The method of calcium analysis, neutron activation analysis, involves the conversion of a portion of ^{48}Ca, a stable isotope, to ^{49}Ca, which is unstable with a half life of 8.72 min, and measuring the characteristic γ radiation emitted. The source of neutrons was the Ford Reactor in the Michigan Memorial Phoenix Project, at the University of Michigan, the pneumatic tube system providing a flux of about 5×10^{12} n cm^{-2} s^{-1}. All the samples, in the milligramme range, were placed in polyethylene vials (cleaned with nitric acid) and freeze dried. They were then irradiated for 1 min, allowed to cool for 3 min, the entire vial was then placed near a 30 cm^3 germanium–lithium detector, FWHM 2.3 keV, connected to a 4,096 channel multichannel analyser calibrated to approximately 1 keV per channel with a CoCs source. Each sample was counted for 400 s. The calcium was measured using the 3,083 keV line of ^{49}Ca with reagent grade $CaCO_3$ powder as the standard. Calculations of the statistical counting errors and variability of controls and standards indicate a possible error of ± 10%. Whether these minute (2 mm) and very thin (30 µm) egg shells were crushed or not, made no difference in the results at this level of analysis.

Figures 1 and 2 show X-ray radiographs of a freshly deposited egg and an egg incubated for 43 d respectively. Both eggs were placed on the same piece of film, exposed and then printed together. Note that whereas the egg shell is made of calcite, the protoconch is made of another polymorph of $CaCO_3$, aragonite. The arrow in Fig. 2 indicates the area in which the embryo has most greatly weakened the egg shell, and at which it will break out. The mechanism of egg shell dissolution and reabsorption is not clear. When the embryo hatches, it consumes any egg shell remnants which are left.

There is evidence that this process of egg shell dissolution occurs in many other families of snails[6]. Calcified eggs have also been reported from land archaeogastropods[7] and from some of those mesogastropods which deposit their eggs on land[8]. It is suggested that land snails evolved the calcified egg shell early in their ancestry. For these terrestrial animals, calcium is often the limiting factor[9]. It would seem to be of great advantage to have the body shell already calcified by the time of hatching, for protection against small predators.

Embryonic absorption of egg shell calcium has been documented in some insects[10], some reptiles and birds[11]. A study of the calcium mobilisation in gastropod embryos with these other groups would be most rewarding from a comparative point of view.

Table 2 Calcium distribution in eggs incubated at 20 °C ± 1

No. of days incubated	mg Ca in egg shell	mg Ca in embryo	%Total Ca in embryo
20	—	0.003*	—
30	0.601	0.126	17
30	0.510	0.190	27
30	0.646	0.126	16
43	0.285	0.366	56
43	0.326	0.218	40
43	0.601	0.126	17
43	0.338	0.246	42
43	0.523	0.146	22
43	0.211	0.346	62
43	0.646	0.126	16
43	0.253	0.302	54
43	0.510	0.190	27
43	0.522*	0.299*	36*

* Ten specimens combined in a single analysis.

I thank Norman E. Kemp and James N. Cather for help, and Jack Jones for assistance with the activation analysis.

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Depressant action of TRH, LH-RH and somatostatin on activity of central neurones

THREE structurally identified hypothalamic-adenohypophyseal regulatory peptides—thyrotropin-releasing hormone (TRH)[1,2], luteinising hormone releasing hormone (LH–RH)[3,4] and growth hormone-release inhibiting hormone (somatostatin or SRIF)[5], have now been localised both in extrahypothalamic regions of the central nervous system (CNS)[6–9] and in certain extraneural regions, such as the pineal gland[10]. There is increasing evidence that these peptides may affect behaviour by direct action on the brain. Systemic administration of TRH to rats induces behavioural effects independent of the pituitary–thyroid axis[11]; LH–RH can induce mating behaviour in hypophysectomised female rats[12], and somatostatin potentiates the behavioural effects of d,l-dopa[13]. These findings together with evidence of high affinity binding sites for TRH in extrahypothalamic tissues[14], identification of LH–RH in synaptosome fractions of brain[15] and localisation of LH–RH[16,17] and somatostatin[18,19] in nerve terminals suggest a role for these peptides in neuronal function. Applying TRH, LH–RH and somatostatin directly to central neurones microiontophoretically, we have found that these peptides have a potent depressant action on the activity of neurones at several levels (cerebral and cerebellar cortex, brain stem and hypothalamus) of the neural axis.

Experiments were performed on male Sprague Dawley rats anaesthetised with urethane (1.25 mg kg^{-1} intraperitoneally in 25% w/v solution) or pentobarbital (50 mg kg^{-1} intraperitoneally with supplemental intravenous doses). In experiments in which extracellular records were obtained from neurones in the cerebral or cerebellar cortices or the cuneate

Table 1 Summary of depressant responses observed from central neurones during microiontophoresis of hypothalamic releasing (or release inhibiting) peptides

	Site	No. tested	No. responsive	Effective current range* (nA)
TRH	Cuneate nucleus	43	12 (28%)	6–20
	Cerebellar cortex	66	28 (42%)	5–30
	Cerebral cortex	36	17 (47%)	15–25
	Hypothalamus (HVM)	50	30 (60%)	10–18
LH–RH	Cerebellar cortex	17	13 (76%)	5–25
	Hypothalamus (HVM)	16	13 (81%)	10–30
Somatostatin	Cerebellar cortex	9 (L)	5 (55%)	7.5–20
		9(C)	5 (55%)	10–25
	Cerebral cortex	13 (L)	11 (84%)	10–30
		15 (C)	12 (80%)	10–30
	Hypothalamus (HVM)	14 (C)	11 (78%)	15–35

All other neurones were unresponsive. Somatostatin was tested in both the linear (L) and cyclised (C) form. HVM, hypothalamic ventromedial nucleus.

* Range of iontophoretic currents which yielded clear depressant effects within 10 s of application. The range of values for either the peptides or the areas does not differ significantly.

nucleus, exposed surfaces were irrigated continuously with mammalian Ringer solution. The hypothalamus was exposed by a retropharyngeal approach, and neurones in the ventromedial nucleus were identified by a characteristic orthodromic response to stimulation of the amygdala or stria terminalis[20]. Single unit activity was recorded with micropipettes filled with 3.0 M NaCl and the frequency of spike discharges, selected with a voltage gate, was monitored on a polygraph. For microiontophoresis, five or seven-barrelled micropipettes were fixed rigidly to the side of the recording micropipette, and the tips were separated by 10–15 μm. Solutions of TRH, LH–RH or somatostatin dissolved in distilled water (5 mM) were placed in microiontophoretic channels and ejected as the cation. Adjacent channels always contained sodium L-glutamate (1.0 M., *p*H 7.0) to excite otherwise silent neurones, and sodium chloride (10 mM) as a current control.

In each area of the CNS studied, only a certain population of neurones responded to microiontophoretic application of individual peptides (Table 1). In responsive neurones, all three peptides depressed discharge frequency (Fig. 1) often in association with an increase in spike size, suggesting membrane hyperpolarisation. For some cells, iontophoretic currents as low as 5–10 nA effected a 50% reduction in spike discharge frequency of both spontaneous and glutamate-evoked activity. The characteristics of the responses in sensitive cells were similar with each peptide: brevity in onset after application of current, rapid recovery after cessation of the ejection current, graded decrease in excitability with increased current, reproducibility with successive applications and lack of similar responses during application of positive control currents (sodium ions). The spike discharge frequency of insensitive neurones showed no consistent change when peptides were applied even with currents as high as 100–160 nA.

Twelve of fourteen peptide-sensitive neurones (six in the cerebellar cortex, four in the cerebral cortex, four in the hypothalamus) were sensitive to both TRH and LH–RH (Fig. 2*b*) although the two remaining neurones were responsive to LH–RH only (Fig. 2*a*). TRH sensitive neurones, more frequent in hypothalamus than elsewhere in the brain, were also found in the cerebellar cortex although this area has a low content of TRH[6] and lacks high affinity TRH binding sites[14]. A wide range of TRH responsiveness for neurones in any one area of the CNS has been reported[21]. As indicated in Table 1, a substantial number of neurones was responsive to LH–RH and somatostatin even in areas where these peptides have either not been detected or are low in concentration. Somatostatin was effective when applied in either the linear or the cyclised (dihydrosomatostatin) form.

Our observation that hypothalamic peptides have potent depressant actions on the excitability of a certain population of neurones in several areas of the CNS extend studies which indicate a depressant action of TRH[21,22] and LH–RH[22,23] when applied to hypothalamic neurones; it seems, however, that this response pattern is not solely restricted to hypothalamic neurones involved in pituitary regulation. This more generalised distribution of peptide-sensitive neurones supports the hypothesis that these peptides are involved in

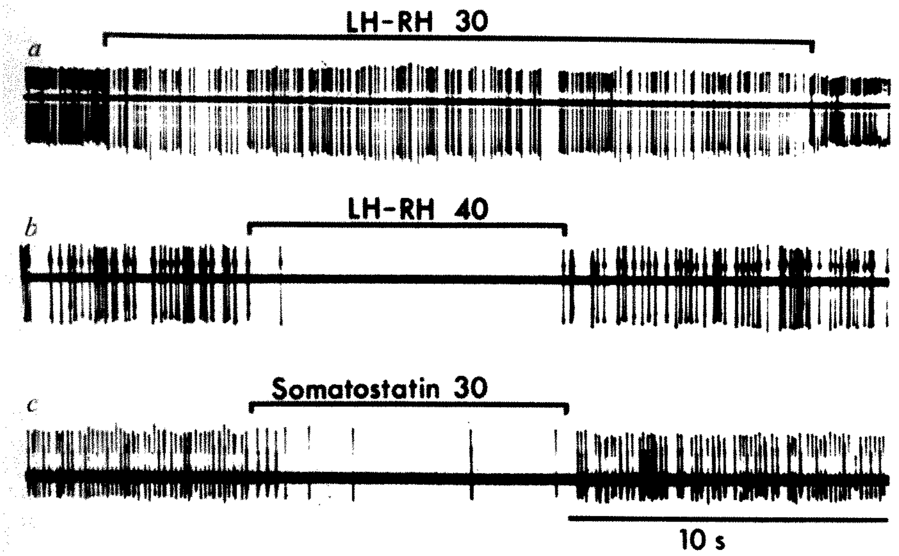

Fig. 1 Representative continuous oscillograph traces to indicate effects of microiontophoresis of LH–RH and somatostatin. Note the increase in spike amplitude (*a*) and decrease in spike discharge frequency which accompanies applications of LH–RH to two hypothalamic neurones located in the ventromedial nucleus (*a*, *b*) and cyclised somatostatin to a neurone in the parietal cortex (*c*). Numbers above each bar indicate the microiontophoretic current (in nA) used to apply each hormone. Time calibration, 10 s.

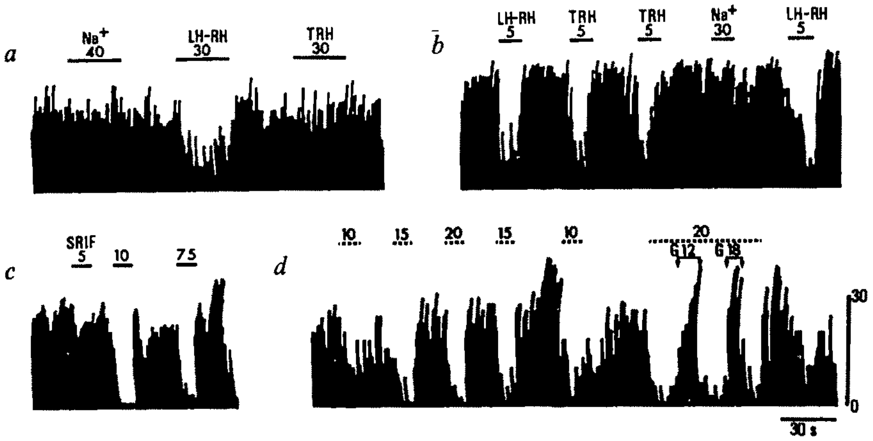

Fig. 2 Polygraph traces of changes in spike discharge frequency of three spontaneously active cerebellar cortical neurones (*a–c*) in response to microiontophoresis of TRH, LH-RH and somatostatin. Cell *a* was responsive to LH-RH but not to either TRH or positive current. The firing frequency of cell *b* was depressed by both TRH and LH–RH. The tracing in (*c*) indicates a neurone the activity of which was depressed by cyclised somatostatin. *d*, Linear somatostatin depressed the spontaneous activity of a parietal cortical neurone. This depression remained stable during longer periods of application and could be overcome by brief applications of L-glutamate (arrows). Vertical calibration bar indicates c.p.s.

the modulation of central neuronal activity. Microiontophoretic studies with substance P, another peptide found in the hypothalamus, indicates a predominantly excitatory action when applied to neurones elsewhere in the brain[24]. Microiontophoretic application of angiotension II was also reported to excite central neurones[25].

An understanding of the role of hypothalamic peptides in neuronal function requires data on their ultrastructural localisation and demonstration of their release from synaptic nerve terminals after stimulation. In support of the latter possibility is recent electrophysiological evidence that axons of certain tuberoinfundibular neurones have two (or more) collaterals, one of which terminates on the portal vessels of the median eminence, while the other either terminates locally within the hypothalamus (anterior hypothalamic–preoptic area) or exits from the hypothalamus to other regions of brain[26]. Thus it is possible that hypothalamic releasing or release-inhibiting peptides are liberated simultaneously both at central synapses as well as at the median eminence terminals of tuberoinfundibular neurones, thereby acting both as neural transmitters and as neurohormones, an earlier suggestion for another hypothalamic peptide, vasopressin[27].

We thank Mr Brian MacKenzie for technical assistance, Mrs M. Walker for typographical aid, Mr W. F. White (Abbott Laboratories, Chicago) for TRH and LH–RH, Dr M. Goetz (Ayerst Laboratories, Montreal) for somatostatin, and the Medical Research Council of Canada for financial support.

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Received February 12, 1975.

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Dopamine depolarising response in a vertebrate neuronal somatic cell hybrid

THERE are various lines of evidence that the catecholamine dopamine (DA) is a neurotransmitter in both invertebrate and vertebrate nervous systems[1–4]. In support of this concept, we report depolarising responses to DA caused by a conductance increase and detected by intracellular recording from a vertebrate somatic cell hybrid of neuronal origin.

Somatic cell hybrids are derived from the fusion of two cell types, producing a dividing cell population that expresses properties contributed by each of the parent cells[5–6]. Hybridisation of cells of neuronal origin has resulted in clonal lines that express several neuronal characteristics[7–9]. We used 108CC-15 (also designated NG108-15), which is a somatic cell hybrid of the mouse neuroblastoma N18TG2 and the rat glioma C6-BU-1. Although one parent was of glial origin, the hybrid has numerous properties of nerve cells such as neurone-like morphology, dopamine-β-hydroxylase activity, choline acetyltransferase activity, dense-core and clear vesicles and sensitivity to acetylcholine[10–12]. Thus for descriptive purposes the hybrid cell is neuronal and can provide data relevant to the role of DA in an organised nervous system. The advantage of 108CC-15 for investigating receptor mechanisms are: (1) it provides a large homogeneous cell population free of the anatomical complexity of organised tissues; (2) there is no extrinsic synaptic input, facilitating study of single units with DA receptors; (3) the cells are in an isolated, controlled environment that can be easily manipulated, and (4) the cells are large (40–70 μm) and intracellular recordings can be made with relative ease.

The average membrane potential of 111 cells was −42.5 mV with an average input resistance of 39 MΩ. Detailed analysis of the electrical properties of this cell line has been presented elsewhere[13].

Figure 1*A* shows the membrane response to graded amounts of DA. Sixty-four per cent of the 111 cells penetrated gave a depolarising response to DA with an associated decrease in resistance (increase in conductance). The rise time of the response varied with the proximity of the DA iontophoretic electrode to the membrane. 3-Methoxytyramine-HCl (Sigma) was also applied iontophoretically so as to rule out a response elicited by nonspecific *p*H effects or a physical stimulus from the current. 3-Methoxytyramine caused no potential or conductance changes in five cells tested but an equal charge of DA gave a response as described above, adding credence to the specificity of the receptor for DA. Repeated applications of DA at short intervals resulted in membrane desensitisation and temporary reduction or loss of the response (Fig. 1*B*). Similar findings have been reported for the excitatory DA response in Aplysia neurones[14]. Iontophoretically applied noradrenaline (NA) depolarised the membrane but the response amplitude was less than that produced by an equal iontophoretic charge of DA. In addition, the membrane became desensitised to DA when NA was applied repeatedly. These results indicated that DA and NA had common receptors, but that DA was more efficacious. Other investigators using 108CC-15 have found that NA and DA both depolarise the membrane (J. Traber, G. Reiser, K. Fischer and B. Hamprecht, personal communication).

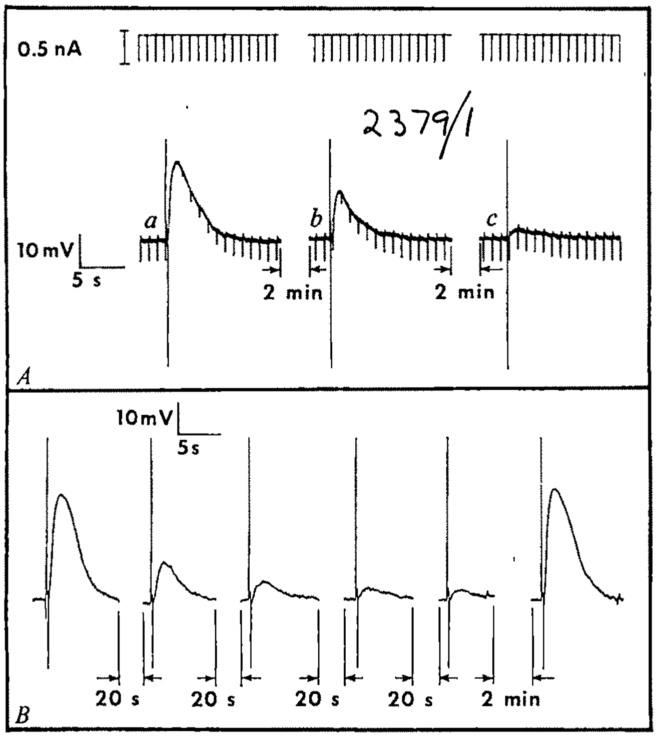

Fig. 1 *A*, DA depolarising response with a conductance increase. Top trace: current pulses passed across the membrane. Lower trace: dose-response characteristic of iontophoretically applied DA obtained by varying the amount of charge delivered. *a*, 600 nC; *b*, 300 nC; *c*, 100 nC. Current pulses indicate the change in resistance. Cells were grown in Falcon dishes with modified F12 medium[18] supplemented with 5% foetal calf serum, hypoxanthine (30 μM increased to 100 μM), aminopterin (0.4 μM) and additional thymidine (3.0 μM increased to 16 μM) at 37 °C and 5% carbon dioxide: 95% air in a water saturated atmosphere. For experiments, 10^4 cells were seeded into 35mm Falcon dishes and grown for at least 7d in medium free of aminopterin but supplemented with 1 mM dibutyryl cyclic adenosine monophosphate (Sigma). The drug induces morphological and biochemical differentiation of neuroblastoma cells in culture[11,19-22]. Standard electrophysiological techniques using a bridge circuit were used to record membrane potential while passing current intracellularly. Glass microelectrodes filled with potassium acetate and potassium chloride and resistances greater than 30 MΩ were used for intracellular recording. For iontophoresis, 1 M dopamine-HCl (Sigma) was dissolved in distilled water and used to fill glass micropipettes (electrode resistance 10–20 MΩ). During recording, culture dishes were maintained at 37 °C and gassed with a stream of water-saturated 10% CO_2: 90% air. *B*, Desensitisation of the receptor by DA. Iontophoretically applied DA was delivered at short time intervals to demonstrate reduction in response amplitude and full amplitude recovery after a longer time interval. Iontophoretic charge was 500 nC.

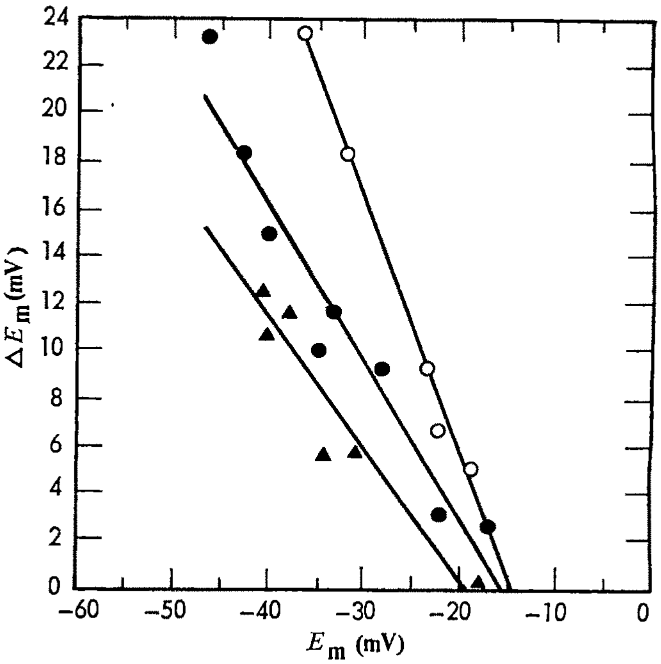

Fig. 2 Reversal potential of the DA response for three cells. Depolarising current was passed across the cell membrane to alter the resting membrane potential. Equal amounts of DA were iontophoretically applied at selected membrane potentials and the response amplitude was determined. Lines were established by linear regression analysis of the data for each cell. The intercept at the abscissa represents the reversal potential.

Figure 2 is a plot of DA response amplitude compared with membrane potential for three individual cells. The intercept at the abscissa was −15.9 mV (±1.2; $n=4$) and represents the DA reversal potential. This value is similar to that found in vertebrate neuromuscular junctions where the acetylcholine response is mediated by an increase in sodium and potassium conductances[15].

Phentolamine, propranolol, chlorpromazine and bulbocapnine blocked the DA response when present in concentrations greater than 50 μM, which agreed with their known central actions. Phentolamine and propranolol are adrenergic blockers. Chlorpromazine is a phenothiazine which interferes with DA receptors[16] and bulbocapnine is a DA antagonist similar in structure to the agonist apomorphine[17].

These studies show that vertebrate cells of neuronal origin can exhibit depolarising responses to DA associated with a conductance increase. Cell preparations of this sort may be of further use in studies of receptor mechanisms in an isolated, controlled environment.

We thank Dr Berndt Hamprecht on whose cell line this study was completed and Drs William Shain and David Carpenter for helpful discussion.

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Received February 4; revised March 24, 1975

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Nerve terminal ATPase as possible trigger for neurotransmitter release

WHEN a wave of depolarisation spreads down an axon and invades the nerve terminals a number of events rapidly occur and it is difficult to establish the one ultimately responsible for the release of neurotransmitter. There is strong evidence to support the contention that calcium ion influx is involved both in the release of acetylcholine[1] and of noradrenaline[2], but ion-exchange processes at the level of the vesicle, not necessarily directly involving calcium have been proposed[3], and the concept of regulation of release through changes in the activity of an adenosine triphosphatase (ATPase) has also been invoked[4,5]. This paper is concerned with the last possibility. Following the inward movement of sodium ions, the inward movement of calcium ions to the enzyme on the inner edge of the nerve terminal membrane would be expected to inhibit the activity of sodium, potassium-activated, magnesium-dependent ATPase (Na,K-ATPase)[6]. Sodium depletion, or the addition of ouabain to preparations *in vitro* would also be expected to inhibit Na,K-ATPase activity. These three conditions of enzyme inhibition have all been shown to result in increased acetylcholine release[4,5].

We have already suggested elsewhere[7–9] that nerve ending particles (synaptosomes) prepared from rat cerebral cortex contain an ouabain-insensitive component of that ATPase activity which is apparently activated by sodium and potassium ions in the presence of magnesium ions, and which is generally assumed to represent Na,K-ATPase. This apparent Na,K-ATPase activity was calculated by subtracting the magnesium-ATPase (Mg-ATPase) activity, determined by measuring the release of inorganic phosphate from Tris-ATP (4 mM) in 50 mM imidazole- HCl buffer *p*H 7.4 containing 5 mM $MgCl_2$, from the total ATPase activity determined in a similar mixture which also contained NaCl (150 mM) and KCl (10 mM). The reaction time was 10 min in each case and sodium dodecylsulphate (0.8%) was used to stop the reaction[9]. The ouabain-insensitive component, the existence of which has recently been supported by others[10,11], is the result of sodium-activated, magnesium-dependent ATPase (Na-ATPase) activity[9], determined by measuring the hydrolysis of ATP in the $MgCl_2$-containing buffer to which NaCl (150 mM) has been added. Note that in synaptosomes, at least, the magnitude of this activity varies with the species of animal, the values for the rat, mouse and guinea pig being 33, 13 and 43% of the apparent Na,K-ATPase activities, respectively.

The implications of the existence of a Na-ATPase together with a true Na,K-ATPase (the ouabain-inhibited enzyme requiring sodium and potassium ions together) in the nerve terminals are considerable if the hypothesis is proposed that inhibition and stimulation of the activities of ATPases are involved in the regulation of neurotransmitter release. We report here data concerning the effects of noradrenaline, phentolamine and acetylcholine on the activities of these enzymes in synaptosomes prepared from rat cerebral cortex as described previously[9].

Noradrenaline (5×10^{-5}–1×10^{-3}M) stimulated both the Na- and the Na,K-ATPase activities of the synaptosomes (Table 1). Other workers[10,11] have also detected stimulation of Na,K-ATPase by noradrenaline and our results indicate that the Na-ATPase is affected to approximately the same extent as the Na,K-ATPase. Moreover, stimulation of the two enzymes by noradrenaline was decreased by approximately the same extent by the α-adrenoceptor antagonist phentolamine (10^{-5} M, lower concentrations being less effective). In direct contrast to the effect of noradrenaline, acetylcholine (10^{-4}–10^{-3}M) inhibited the Na,K-ATPase but the activity of the Na-ATPase was not altered in the conditions used.

Table 1 Effects of noradrenaline, phentolamine and acetylcholine on ATPase activities of synaptosomes prepared from rat cerebral cortex

Drug	Concentration (M)	Relative ATPase activities* NaK	Na
None		100	100
Noradrenaline	1×10^{-6}	$107.0\pm3.9(5)$	$96.1\pm6.4(5)$
	5×10^{-5}	$132.5\pm2.5(2)$	$135.0\pm6.9(2)$
	1×10^{-4}	$141.3\pm3.0(3)$	$136.3\pm16.3(3)$
	1×10^{-3}	$145.2\pm5.9(5)$	$139.3\pm10.0(5)$
Phentolamine	1×10^{-5}	$100.0\pm2.0(2)$	$97.4\pm1.7(2)$
Phentolamine Noradrenaline	1×10^{-5} 5×10^{-5}	$99.8\pm2.6(2)$	$102.4\pm2.4(2)$
Acetylcholine	1×10^{-5}	86.8 (1)	97.5 (1)
	1×10^{-4}	$58.5\pm2.6(2)$	$100.9\pm3.4(2)$
	1×10^{-3}	$60.1\pm0.7(3)$	$97.1\pm6.7(3)$

Numbers of observations in parentheses.

*Results expressed as percentages of control values typically $6.4\pm0.5(21)$ and $3.3\pm0.3(22)$ μmol inorganic phosphate per mg protein per h for the Na,K- and the Na-ATPases, respectively. Percentage values under the different conditions have been calculated by comparison with paired controls of the same series.

These results should be considered in the light of evidence suggesting that noradrenaline released from the nerve terminal can cause feedback inhibition of further release[12,13]. Nerve terminal ATPases may be involved in this mechanism. Thus, although inhibition, of Na,K-ATPase by calcium ions, for instance, may increase neurotransmitter release, stimulation of this enzyme, or of a closely related one, may depress it. The effect of phentolamine in preventing the stimulation of both the Na,K-ATPase and the Na-ATPase by noradrenaline is then compatible with the proposition that feedback inhibition is mediated through α-adrenoceptors[12,14].

Although stimulation of both Na- and Na,K-ATPase could be involved in noradrenaline-mediated depression of noradrenaline release, any modulation of this release at synapses by acetylcholine is likely to be through effects on Na,K-ATPase alone (Table 1), inhibition of the enzyme presumably increasing noradrenaline release. Whether or not inhibition of Na,K-ATPase by acetylcholine should be viewed in the light of the suggestion of Langer[15] that presynaptic nicotinic and muscarinic receptor activation can influence noradrenaline release, or in the light of the cholinergic link hypothesis of Burn and Rand[16] is a matter for conjecture; but at least the change in the activity of the enzyme is in the right direction to permit reconciliation with these ideas as well as with the better documented evidence linking increased neurotransmitter release with such factors as calcium ion entry.

Modulation of neurotransmitter release is also believed to be an important function of prostaglandins. Hedqvist[17] showed that the noradrenaline release associated with electrical stimulation of nerves supplying the perfused cat spleen was inhibited by prostaglandins of the E series and he later found that the inhibitory effect of prostaglandin E_2 (PGE_2) was antagonised by calcium ions[18]. We have observed[9] that PGE_1 stimulates synaptosomal Na,K-ATPase activity. Here again it could be assumed that stimulation of the Na,K-ATPase inhibits neurotransmitter release, and that the inhibitory effect of calcium ions on the activity of the enzyme antagonises this process.

Evidence for a more direct association between the activities of ATPases in the nerve terminals and the regulation of neurotransmitter release should be obtained before the hypothesis is strongly supported. The mechanism of regulation by the enzymes requires consideration although one could propose that a change in the local environment of a membrane-bound enzyme results in both a change in its activity and its conformation such that an increase in exocytosis occurs. It could be argued, however, that the rates of activity of the enzymes are not sufficiently high to regulate neurotransmitter release to a fine degree by such a mechanism. Again, the observations mentioned here in connection with the hypothesis are drawn from work involving both peripheral nerves and the central nervous system, and the information concerning the effects of drugs on the activities of the ATPases has come from work with cerebral cortex synaptosomes which contain at the least both cholinergic and adrenergic types. We feel, nevertheless, that the hypothesis merits further attention.

We thank Mrs Lorna Smith for assistance.

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Received February 4, accepted April 4, 1975.

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Oxygen control of cytochrome *c* oxidase synthesis in isolated mitochondria from *Saccharomyces cerevisiae*

DURING anaerobic growth *Saccharomyces cerevisiae* cells produce respiratory-deficient mitochondria-like structures—promitochondria—which contain a normal complement of mitochondrial DNA (mtDNA) and many mitochondrial enzymes but which lack cytochrome components of the inner mitochondrial membrane[1–4]. On exposure to oxygen these respiratory-deficient structures develop a full complement of cytochromes and differentiate into respiring mitochondria[5]. Although the precise mechanism by which oxygen mediates mitochondrial biogenesis is still incompletely understood recent studies with whole yeast cells have shown that one effect of oxygen is at the level of either synthesis or assembly of some mitochondrial translation products[6–8]. Unfortunately, these *in vivo* studies could not localise the primary effect of oxygen to the mitochondrion nor could they demonstrate that oxygen acts directly on mitochondrial protein synthesis rather than indirectly by inhibiting other biochemical reactions, for example in the biosynthesis of haem[9], required for the development of functional cytochromes. As a first step towards localising the effect of oxygen and ascertaining if it is indeed at the level of the mitochondrial protein synthesis we have studied the effect of O_2 on the *in vitro* synthesis of three mitochondrial translation products, subunits I, II and III of cytochrome *c* oxidase, by isolated mitochondria. We have found that mitochondria incubated in the presence of oxygen synthesise subunits I, II and III and assemble them with the four cytoplasmically-made subunits[8] into a holoenzyme[10], although mitochondria incubated in the absence of oxygen do not synthesise subunits I and II. These results demonstrate that oxygen acts directly on the mitochondrion and strongly suggest that at least one step in mitochondrial transcription-translation requires oxygen.

Table 1 Effect of anaerobiosis on the incorporation of ^{14}C-leucine into isolated mitochondria

Additions or omissions	Incorporation (c.p.m. per mg protein) Aerobic	Anaerobic
None	3,500	2,680
+ Chloramphenicol	314	258
+ Cycloheximide	3,550	—
− ATP, PEP, pK	—	1,000

Protein synthesis in isolated mitochondria was measured as described previously[10]. Anaerobic incubations were carried out in long test tubes with the reaction mixture covered with liquid paraffin. Five minutes after the oxygen was consumed by the respiring mitochondria (usually in 1–1.5 min as checked with a Clark oxygen electrode in a parallel incubation) the reaction was started by the addition of 0.2 µCi ^{14}C-leucine (60 mCi mmol^{-1}) per ml by means of a syringe fitted with a long needle.

Table 1 summarises the effect of anaerobiosis on the incorporation of radioactive leucine into acid-insoluble material by isolated mitochondria. Both aerobic and anaerobic incorporation show the expected response to chloramphenicol and cycloheximide, indicating that essentially all of the protein synthesis is taking place on mitochondrial ribosomes. Incorporation of radioactive leucine into protein in anaerobic conditions is dependent on the continuous supply of ATP by the ATP-regenerating system, phosphoenolpyruvate–pyruvate kinase, and is 50–70% (4 experiments) of that incorporated in aerobic conditions.

Table 2 Effects of anaerobiosis on the labelling of cytochrome *c* oxidase

Labelling condition	Experiment	Specific activity of labelled mitochondria (c.p.m. per mg protein)	Total mitochondrial label immuno-precipitated (%)
Aerobic	1	4.85×10^4	8.7
	2	3.33×10^5	10.4
Anaerobic	1	2.85×10^4	4.2
	2	1.59×10^5	6.0

Isolated mitochondria were pulse-labelled with 0.1 mCi ml^{-1} of L-4,5-^{3}H-leucine (3Ci mmol^{-1}) for 20 min either aerobically or anaerobically as described in Table 1. The labelled mitochondria were solubilised with cholate-KCl and subjected to immunotitration using an antiserum to holo-cytochrome *c* oxidase as described previously[10]. Values for the percentage of total mitochondrial label immunoprecipitated represent plateau values taken from immunotitration curves and have been corrected for nonspecific precipitation obtained with control serum.

The effect of oxygen on the *in vitro* synthesis of subunits I, II and III of cytochrome *c* oxidase was assessed by determining the amount of radioactivity precipitated from mitochondrial extracts by an antiserum to cytochrome *c* oxidase and analysing the immuno-precipitates by sodium dodecyl sulphate (SDS) gel electrophoresis. The immunotitration results shown in Table 2 indicate that labelling of cytochrome *c* oxidase in anaerobic conditions is reduced by about 50% relative to the aerobic control. Electrophoretic analysis (Fig. 1*a* and *b*) of the immunoprecipitates from aerobically and anaerobically-labelled mitochondria reveals that subunit III of cytochrome *c* oxidase is labelled both in the presence and absence of oxygen, but that subunits I and II are labelled only in the presence of oxygen. Since subunits I and II account for 40–50% of the radioactivity in immunoprecipitates from aerobically-labelled mitochondria it seems that the decreased level of anaerobic incorporation is caused solely by the lack of synthesis of these two subunits. This finding that subunits I and II are synthesised *in vitro* only in the presence of oxygen fully parallels the *in vivo* studies of Mason and Schatz[8] and, in addition, demonstrates that oxygen can act directly on the mitochondrion.

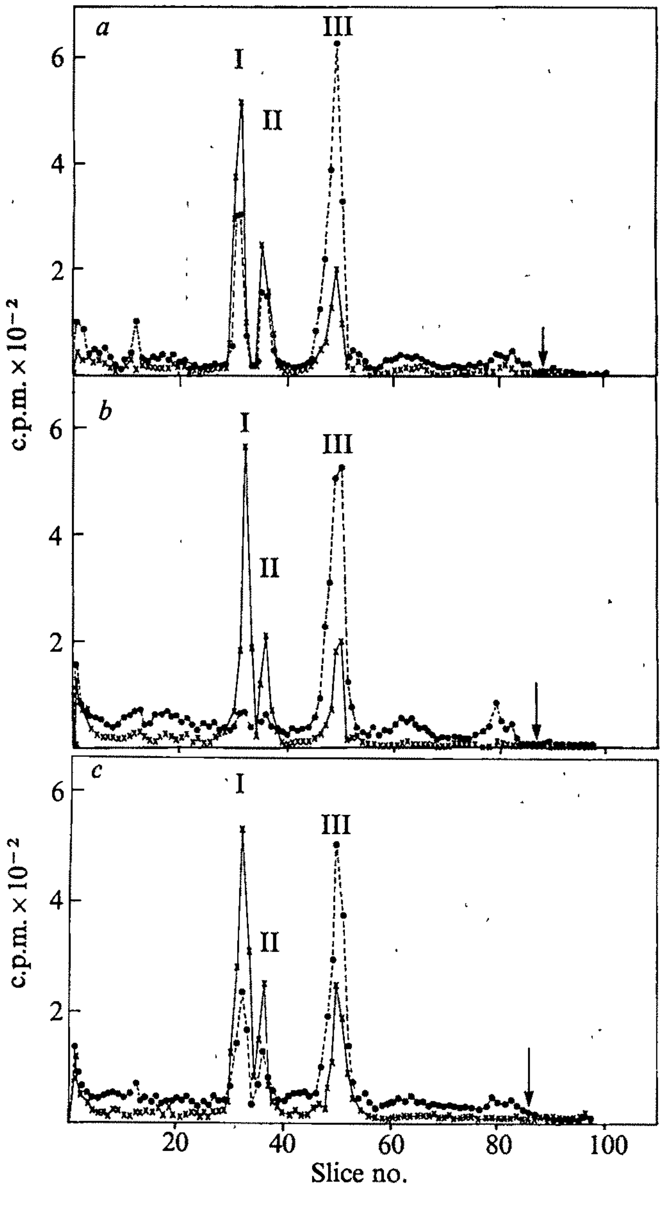

Fig. 1 SDS polyacrylamide gel analysis of cytochrome *c* oxidase immunoprecipitates from extracts of mitochondria labelled: *a*, aerobically; *b*, anaerobically; and *c*, aerobically after a 20 min anaerobic preincubation. Isolated mitochondria which were labelled *in vitro* with ^{3}H-leucine either aerobically or anaerobically were solubilised in cholate-KCl, mixed with mitochondrial extracts from cells labelled *in vivo* with ^{14}C-leucine in the presence of cycloheximide and subjected to immunoprecipitation[10] using an antiserum to holocytochrome *c* oxidase. The immunoprecipitates were solubilised in SDS and electrophoresed on 12% acrylamide–0.375% *bis*-acrylamide gels as described previously[13]. The arrow marks the position of the dye front. ×, ^{14}C-leucine counts immunoprecipitated from *in vivo* labelled mitochondria (cells + cycloheximide); ●, ^{3}H-leucine counts immunoprecipitated from *in vitro* labelled mitochondria.

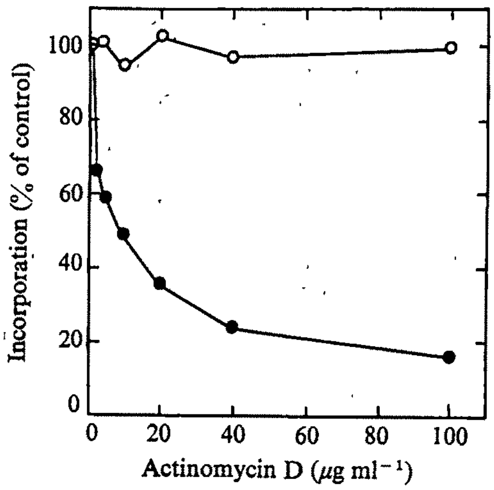

Fig. 2 Effect of actinomycin D on RNA and protein synthesis in isolated mitochondria. Mitochondria (2 mg) were incubated as described previously[10] and pulse-labelled for 20 min with 50 μM ^{3}H-UTP (10^6 c.p.m. nmol^{-1}) and 5 μM ^{1}-C-leucine (0.04×10^6 c.m.p. nmol^{-1}). Acid-insoluble counts were measured. The 100% value corresponded to 5 pmol ^{1}-C-leucine (○) and 0.5 pmol ^{3}H-UTP (●) incorporated per mg protein per min.

To exclude the general deleterious effect of the absence of oxygen on mitochondrial protein synthesis, we have tested the reversibility of the oxygen effect. To reproduce *in vitro* the "aerobic adaptation" phenomenon already well documented from *in vivo* studies we incubated isolated mitochondria anaerobically for 20 min and then aerated them in the presence of ^{3}H-leucine. A comparison of Fig. 1(*c*) with Fig. 1(*a*) reveals that subunits I and II are labelled to near normal levels after the anaerobic preincubation. Thus, it seems that the response of isolated mitochondria to the absence of oxygen is exactly like that of whole cells.

At the moment there seem to be three possibilities to explain the finding that subunit III of cytochrome *c* oxidase is unresponsive to oxygen although subunits I and II are positively controlled by it. First, oxygen may act at the level of mitochondrial transcription either by stimulating an RNA polymerase[11] or by inactivating a repressor[12]. This explanation assumes that the genetic information for subunits I and II of cytochrome *c* oxidase is present on mtDNA, an assumption for which there is no direct evidence. Second, oxygen may act at the level of mitochondrial translation, and third, oxygen may be required for the integration of polypeptide subunits (for example subunits I and II of cytochrome *c* oxidase) into enzyme complexes. For this explanation to be correct we would have to assume that subunits which are not integrated are rapidly degraded as they cannot be detected by immunoprecipitation. Since we have not observed a substantial increase in low molecular weight polypeptides in immunoprecipitates from anaerobically-labelled mitochondria we consider this explanation unlikely. While we cannot rule out the first possibility, some evidence against it is obtained from Fig. 2. Actinomycin D in a rather high concentration seems to be an effective inhibitor of mitochondrial transcription (inhibiting UTP incorporation up to 80%), whereas at the same concentration no effect is seen on the incorporation of leucine. This result indicates that mitochondrial translation is not dependent on at least the bulk of mitochondrial transcription. It seems unlikely, therefore, that inhibition of mitochondrial transcription would lead to the observed inhibition of incorporation of label into subunits I and II of cytochrome *c* oxidase. Thus, at the moment, it seems most likely that oxygen is regulating the synthesis of subunits I and II of cytochrome *c* oxidase directly at the level of mitochondrial translation.

We thank Mrs N. van Harten-Loosbroek and Mrs J. Kavanagh for technical assistance, and Dr P. Borst for discussions. This work was supported by a research grant (to R.O.P.) from the Connecticut Research Foundation and by a grant (to P.B.) from The Netherlands Foundation for Chemical Research with financial aid from The Netherlands Organisation for the Advancement of Pure Research.

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Molecular basis for the α_1-protease inhibitor deficiency

THE α_1-protease inhibitor (formerly α_1-antitrypsin) is inherited by a series of autosomal codominant alleles that determine its concentration and electrophoretic mobility. Serum from individuals with severe α_1-protease inhibitor deficiency usually contains an electrophoretically slow-moving α_1-protease inhibitor (type ZZ) in an amount 10–40% of that found in the normal population (type MM). This deficient state is associated with either an early-onset form of familial emphysema or familial infantile cirrhosis[1].

So far, however, the molecular basis for this deficiency has not been established although Bell and Carrell[2] and Cox[3] suggested that the Z protein lacked some of the sialic acid residues found in the normal protein. They reasoned that a glycoprotein with fewer terminal sialic acid residues could be expected to accumulate intracellularly by binding to the plasma membrane of liver cells, thus effectively decreasing the concentration of the circulating protease-inhibitor. This hypothesis is consistent with a report[4] of patients with severe α_1-protease inhibitor deficiency (type ZZ) whose serum was markedly deficient in sialic acid transferase activity. We have now found that the Z protein not only contains less sialic acid but also contains smaller amounts of the other carbohydrates than the M protein. Based on the established structures of the two oligosaccharide units present in the M protein, it seems that there are four oligosaccharide units in the M protein and three in the Z protein. We have also observed that the amino acid composition of the Z protein has a higher amount of glycine and arginine than that of the M protein. It is possible that this difference in amino acid sequence affects the attachment of the carbohydrate side chains, leading to a difference in the rate of secretion of the M and Z proteins which is ultimately responsible for the low concentration of the circulating Z protein in individuals with severe α_1-protease inhibitor deficiency.

The α_1-protease inhibitor was isolated from individuals of genetic type MM and ZZ and purified as before[5] and the genetic typing[6] was determined by two-dimensional electrophoresis. Since no change in the electrophoretic pattern of either protease inhibitor was observed before or after purification, the procedures for isolation did not alter the protein molecule. The isolated products were free of albumin and orosomucoid as shown by immunodiffusion on agar plates, and were homogeneous when subjected to disc electrophoresis on polyacrylamide gel[7] at *p*H 8.9 or 7.0. Results to be published in full elsewhere show that the M protein has a molecular weight of 54,000 and consists of a single polypeptide chain with four carbohydrate side chains of two structural types. The first type consists of four residues of N-acetylglucosamine, three of mannose, three of galactose and three of sialic acid. The second type consists of three residues of N-acetylglucosamine, three of mannose, two of galactose and two of sialic acid. These two types of oligosaccharide unit are present in equal amounts in the M protein.

Table 1 Comparison of the composition of amino acids and carbohydrates of the M and the Z α_1-protease inhibitor

	mol per 54,000 of protein	
	M	Z
Aspartic acid	43	42
Threonine	33	29
Serine	27	27
Glutamic acid	54	51
Proline	19	19
Glycine	22	30
Alanine	27	27
Valine	24	24
Half-cystine	ND	ND
Methionine	9	9
Isoleucine	18	16
Leucine	44	43
Tyrosine	6	6
Phenylalanine	28	26
Lysine	37	33
Histidine	12	13
Arginine	8	12
Tryptophan	ND	ND
N-acetyl-D-glucosamine	13	11
D-mannose	12	8
D-galactose	9	6
Sialic acid	9	5

Samples of α_1-protease inhibitor were hydrolysed in the following conditions: for amino acids, 6 N HCl at 110 °C *in vacuo* for 48 h; for N-acetyl-D-glucosamine, 4 N HCl at 100 °C *in vacuo* for 6 h; for neutral sugars, D-galactose and D-mannose, 1 N H_2SO_4 in sealed tubes for 8 h at 100 °C and for sialic acid, 0.18 N H_2SO_4 at 80 °C for 1 h. Amino acids and D-glucosamine were analysed on a Technicon automated amino acid analyser[10], whereas the neutral sugars were determined on an automated sugar analyser according to Lee *et al.*[11]. Sialic acid was determined as described by Warren[12]. Norleucine was used as an internal standard for analysis of amino acids and D-glucosamine, and D-xylose was used for D-galactose and D-mannose. ND, not determined.

Table 1 shows that the mannose content in the Z protein is approximately three-quarters of that of the M protein and suggests that the Z protein contains three instead of four carbohydrate side chains. Indeed, the Z protein contains roughly four sialic acid residues fewer than does the corresponding M protein. This result is consistent with the speculation of Bell and Carrell[2] and Cox[3]. The difference in sialic acid residues, however, does not necessarily explain the low concentration of the α_1-protease inhibitor in circulation since it is the exposure of the non-reducing terminal galactosyl residues that provides a means by which the liver recognises and removes asialoproteins[8,9]. Thus, even though the M and Z proteins differ in the number of galactosyl and sialic acid residues, as we have shown, the number of exposed galactosyl residues between the two proteins is essentially the same. If, however, the efficiency in the secretion of liver protein depends on the number of carbohydrate side chains, it is conceivable that the extra carbohydrate chain present in the M protein facilitates its secretion and thus maintains its concentration at the normal level.

Table 1 also compares amino acid composition between the M and Z protein. Because of the relatively large molecular weight of the protein, the data are more difficult to interpret. There was minimal difference (±10%) between the two homologous proteins, except that the Z protein had a significantly larger amount of argininyl and glycyl residues. In this case, the M protein would have been expected to be more negatively charged than the Z protein. This is verified by comparing the electrophoric mobilities of these two proteins and their corresponding asialoproteins. The mobility of the Z protein toward the anode was considerably slower than that of the M protein. After the removal of the sialic acid residues by digestion of the proteins with neuramidase (5% by weight) at 38 °C for 16 h, the electrophoric mobilities of both proteins were retarded, but the asialo Z protein maintained a slightly slower mobility than the asialo M protein, consistent with the amino acid composition data shown in Table 1.

This research was supported by a grant from the National Institutes of Health. We thank Drs R. Talamo and J. Pierce for performing the electrophoresis in their laboratories and for providing serum from ZZ individuals.

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Received January 27; revised April 2, 1975.

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Non-random occurrence of 7–14 translocations in human lymphocyte cultures

SOMATIC cells dividing *in vitro* occasionally show chromosome recombination between non-homologues, most often in situations associated with an increased frequency of chromosome breakage and reunion[1,2], their occurrence being recognised by the observation of exchange figures. With the advent of new techniques for chromosome identification[3], it is now possible to identify the chromosomes involved in these rearrangements and to recognise some translocations which might otherwise be missed.

We have observed about 5,000 trypsin-banded[4] metaphases, from 250 individuals, among which a total of 9 cells seem to have a *de novo* balanced translocation. (The 'true' denominator needed to determine the percentage of abnormal cells of this type is necessarily imprecise, since in the course of scanning a preparation, attention may be drawn to a somewhat imperfectly banded metaphase with a morphologically abnormal chromosome and/or a unique banding pattern.) These translocations seem to be non-random, since three of the nine abnormal cells involved a translocation between chromosomes 7 and 14 (Fig. 1). These three cells were noted in three different individuals, studied for disparate reasons: one had Rubinstein–Taybi syndrome, one had multisystem nonspecific congenital anomalies and one was the healthy father of an infant with trisomy 22.

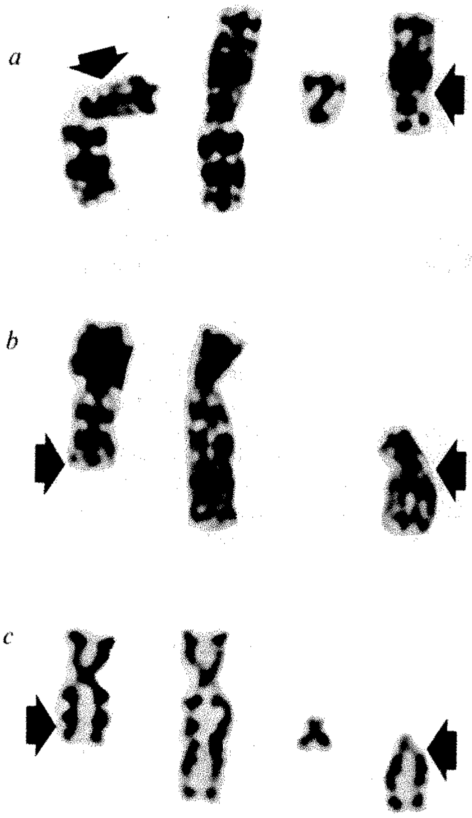

Fig. 1 Partial karyotypes of the three individuals found to have 7–14 translocations. The arrows indicate the breakpoints believed most likely for the number 7 and 14 chromosomes (see text). Note that there was no residual small acrocentric present in cell *b*. (Cell *c* was noted after routine Giemsa staining: it was subsequently destained and trypsin banding carried out.) The chromosomes have been arranged to match homologous segments whenever possible.

All three 7–14 translocations arose in short term (72-h) lymphocyte culture and the breakpoints of the number 14 chromosomes all seemed to involve the same region, q1(1–2). The breakpoints of the number 7 chromosomes were more variable (Fig. 1); cell *a* being 7p13; *b* 7q3(3–6); *c* 7q3(4–6). In two of the other six translocation lymphocytes noted in this study, a 7 or 14 chromosome was also involved; these were 46,XY, t(2;14)(p11;q32) and 46,XX, t(7;10)(q11–22; q2(4–6)). None of the preparations containing a *de novo* translocation showed any suggestion of an increased frequency of chromosome breakage.

It seems clear from these results that translocations between chromosomes 7 and 14 arise much more frequently during *in vitro* culture than would be expected on a random basis. This finding is corroborated by results in the two following papers[5,6] in this issue. The culture time allows for the possibility that the breakage and reunion may be an *in vitro* phenomenon only, and although the circumstances suggest that it is not likely to be a result of mycoplasma infection, a viral aetiology cannot be excluded.

The breakpoints involved in somatic *de novo* 7–14 translocations seem to be relatively specific. Combining these data with those of Beatty-DeSana *et al.*[5] and Hecht *et al.*[6], we note that the 14q1(1–2) region is involved in all twelve somatic 7–14 translocation cells observed, together with the 7q3(3–6) region in six of these and the 7p13 region in the remainder. None of these breakpoints occurred in the 2–14 and 7–10 single cell translocation noted above. Further, although chromosome 14 is commonly involved in human meiotic translocations[7-9], meiotic 7–14 translocations seem to be uncommon and those reported previously[10,11] involve breakpoints other than those above.

Our observations could be a result of an increased tendency to breakage in certain regions of chromosomes 14 and 7, together with the relative proximity of these chromosomes during somatic replication. The latter may be associated with the residua of ancient homology between heterologous chromosomes[12] and we think that there is some resemblance between the 14q and 7p banding patterns. This theory would suggest an increased number of 7–14 exchanges following lymphocyte irradiation, however, but this does not occur[13-15].

Similar 7–14 translocations occurring in proliferative clones[6] have been noted in ataxia-telangiectasia[1,6], a condition often associated with lymphoid neoplasia. Because of the rarity of meiotic 7–14 translocations and the known association of another relatively specific somatic translocation with chronic myeloid leukaemia[16], we wonder whether these observed 7–14 somatic translocations may also have an increased malignant propensity.

This study was assisted by funds provided by a Public Health Research Grant of the Canadian National Health Grants program.

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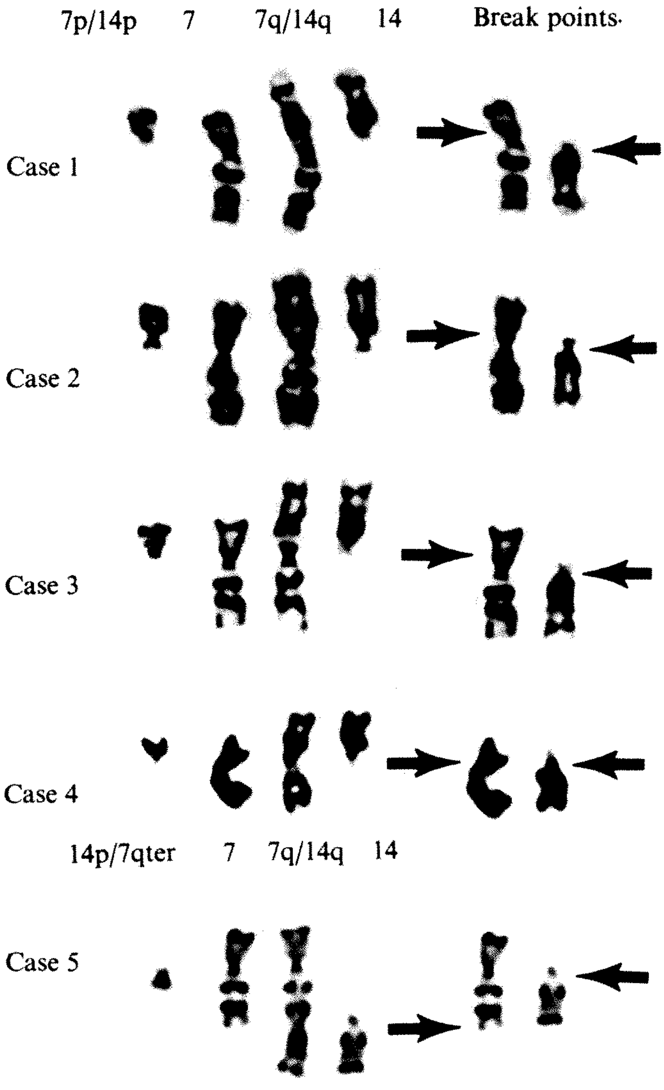
CYTOGENETIC studies of human lymphocytes cultured from patients and their families using known banding methods [1-2] provide an opportunity to observe spontaneous chromosomal aberrations, to identify the chromosomes involved in a rearrangement and aid in interpreting the cytogenetic events leading to their formation. In our investigations of many high risk families over a 4-yr period, all breaks, fragments and rearrangements noted in the lymphocyte cultures were recorded, along with the total number of cells counted because of previous experience in the study of X-ray induced chromosomal aberrations[4,5]. The observations reported here are from 72-h cultures, which may complicate their interpretation[6,7] since they are subject to the selective advantages or disadvantages of a particular chromosomal rearrangement and other factors. The many variables inherent in the analysis preclude rigorous statistical treatment of these data, but a general description of the nature of occurrence may be helpful.

Studies of 25 lymphocyte metaphases each are from 852 consecutively karyotyped mentally retarded people and their families, together with others serving as controls in various studies. Five unrelated people aged less than 40 with normal karyotypes (three with nonspecific mental retardation and two normal, first-degree relatives of people with chromosome anomalies) were found to have a single cell containing a reciprocal translocation between chromosomes 7 and 14. The apparent break point was at 14q1(1–2) in all five, and at 7p13 in four and 7q3(4–6) in the fifth (Fig. 1)[8]. Amplification of the pale band at the region where the two breaks are thought to have joined in the large translocation chromosome indicates that the break is at the edge of the pale band in each chromosome, so that region 7p13 (or 7q3(4–6) in one translocation) is now adjacent to 14q11. We found a total of 42 two-break rearrangements (1 in 500 cells) including dicentrics and rings—usually with accompanying fragments—translocations and exceedingly rare chromatid exchanges. With the exception of the 7–14 translocation, no other consistent aberration was found. Three others, however, involved D-group chromosomes, two dicentrics with fragments, and a 13–14 translocation, all three of which had breaks seemingly in the 14q11 and telomere regions. The consistent translocations have been found once every 8–10 months during the past 4 yr. No infection of cultures or contaminant of the culture media is suspected.

A sample of 25 cells from a single human represents only 2×10^{-9} of the total lymphocyte population[9]; thus the nonrandom occurrence of a 7–14 translocation in a single cell of five individuals, not known to have any malignancy or any

Fig. 1 Sporadic reciprocal translocations involving chromosomes 7 and 14 in human lymphocytes.



suggestion of a predisposition to increased chromosome breakage, indicates that this may be a phenomenon more widespread in routine lymphocyte cultures than is now known. Studies from other cytogenetics laboratories reported in this issue[10,11] provide similar findings which confirm these results. The similarity of these aberrant chromosomes indicates a specificity of breakage points which suggests that their presence is significant and that one or more of the following factors may be involved: A specific aetiological agent[12]; some selective advantage[13] conferred by the translocation; a genetically-based alteration of the repair mechanism[14]; or the spatial arrangement of the chromosomes in the cell. The true significance of these translocations awaits further study.

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Received December 4, 1974; accepted April 3, 1975.

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WE report here the occurrence of somatic chromosome translocations in lymphocytes from individuals studied for clinical cytogenetic diagnosis. We note that these translocations are non-random in composition and resemble those in ataxia-telangiectasia (A-T) lymphocyte clones[1]. Our data have been collated with information from two other centres (Halifax and Atlanta) reported in this issue[2,3], to provide a preliminary estimate of the frequency of chromosome translocations in cultured lymphocytes.

Our sample consisted of 6,000 lymphocyte metaphases; 20 from each of 300 consecutive patients or relatives referred for genetic counselling. None was known to have a chromosome instability syndrome, malignancy, or unusual X-ray or drug exposure. Lymphocytes were cultured with phytohaemagglutinin (PHA) for 68–72 h and harvested with colchicine. Metaphases were Giemsa-banded, selected under low power microscopy and photographed. Karyotypes were prepared from two or more cells per person[4].

Four of the 6,000 cells were found to contain a chromosome rearrangement and each of the four was from a different person. Each rearrangement was a translocation between chromosome 7 and 14 (Fig. 1). Three involved the q33–35 region and one the p13 band of chromosome 7. The breakpoints in chromosome 14 were consistently in band q12.

The chromosome rearrangements in this study resemble those observed in A-T lymphocytes. Lymphocyte clones from 22 A-T patients have been detected, each with a translocation. These A-T translocations involve a D chromosome, identified as No. 14, with the breakpoints in band 14q12. The other chromosome in the A-T translocation is often a No. 7 with the breakpoints (ref. 1 and B.K.M., F.H., D. G. Harnden and R. Teplitz, ref. 8) in p13 or q33-35, as in our study. These data suggest that in lymphocyte somatic chromosome rearrangements there is non-random involvement of chromosomes 7 and 14.

Table 1 Frequency of translocations in cultured human lymphocytes

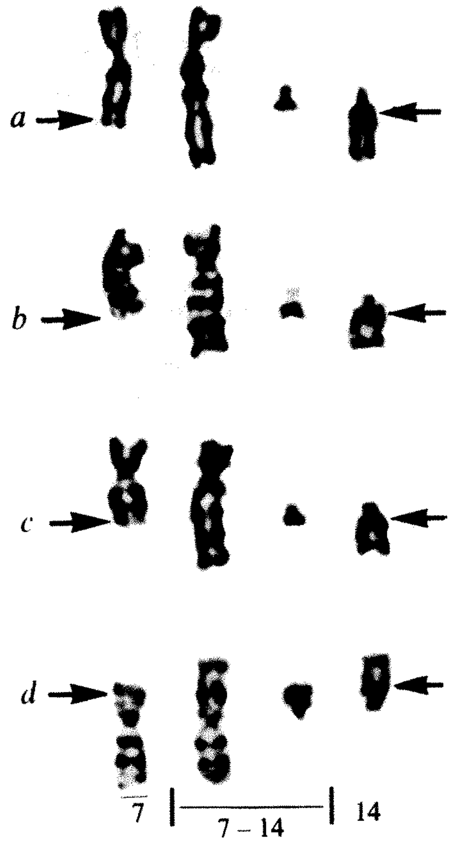
Locale	Individuals	Lymphocytes	All translocations No. (freq.)	7–14 translocations No. (freq.)
Halifax	250	5,000	9 (1/556)	3 (1/1667)
Portland/Denver	300	6,000	4 (1/1500)	4 (1/1500)
Atlanta	852	21,300	42 (1/507)	5 (1/4260)
Total	1,402	32,300	55 (1/587)	12 (1/2692)

From our study and those reported in this issue (refs. 2,3 and Table 1) the frequency of *de novo* chromosome translocations in cultured lymphocytes is calculated to be roughly 1.7×10^{-3}. The frequency of 7–14 translocations is roughly 4×10^{-4}. These are crude estimates, since the sample size is relatively small and subtle rearrangements may have been missed.

By comparison, it is interesting that the mutation rate for structural autosomal rearrangements in germ cells resulting in live births has been estimated to be in the same range, about 4×10^{-4} (ref. 5). It is also interesting that meiotic translocations in humans are generally non-random in composition. For example, D/D translocations are largely of the 13–14 type[7] and D translocations in patients with Down's syndrome tend preferentially to be of the 14–21 type[6,7].

The basis for the non-randomness of somatic chromosome rearrangements is open to conjecture. It must reflect a preferential pattern of rearrangement and/or selection for lymphocytes with 7–14 translocations. The repositioning of genetic material ('position effect') presumably may alter the expression of genes and thus change cell behaviour and function in some as yet unexplained manner.

Fig. 1 Translocations in lymphocytes cultured from four individuals (*a*–*d*). The arrow on the left indicates the point at which the distal part of chromosome 14 is translocated to the long arm (*a*–*c*) or to the short arm (*d*) of chromosome 7. The arrow on the right indicates the breakpoint in chromosome 14.



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The biological meaning of these translocations in lymphocytes is not yet clear. They may occasionally we believe (B.K.M., F.H., D. G. Harnden and R. Teplitz, ref. 8), represent an early tentative step towards the eventual development of lymphoid malignancy.

This work was supported by grants from the National Institute of Child Health and Human Development and the National Cancer Institute and an NICHD postdoctral research stipend (to B.K.M.).

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Factor suppressing α-foetoprotein production in newborn mice

α-FOETOPROTEIN (AFP) is one of the main proteins of embryonic serum. Its synthesis begins in the yolk sac, continues in embryonic liver and terminates, in mice and rats, at the beginning of the postnatal period[1,2]. Presumably, a powerful suppression factor acts on AFP production by embryonic liver cells after the embryonic period has finished. The present study was undertaken to determine this factor(s).

Families of newborn BALB/c mice were divided into two groups. One group was injected daily with serum or extracts of various organs of syngeneic adult mice and embryos, and the other was untreated. After 10 d, the AFP titre was determined in the serum of each group of newborn mice. Extracts of small intestine were used because, during embryogenesis, the liver is formed out of the small intestine and various endocrine connections may persist between these two organs.

Extracts (see Table 1) were injected into the skin of the back of newborn mice, the volume of the material injected increasing as the animals grew older. From day 1 to the day 4 of life, mice were inoculated with 0.1 ml, on days 5 and 6 with 0.2 ml, on day 7 with 0.3 ml, on days 8 and 9 with 0.5 ml. Newborn mice were injected, according to the same schedule, with sterile serum from adult mice. Each mouse killed on the day 10 had received 2.1 ml 20% extract. The AFP titre of controls receiving only Hanks' solution was not affected (Table 1).

Microprecipitation in agar was performed as described previously[3]. Mouse serum was diluted from 1:2 to 1:1,024. This test system (1:2) was used with the above dilutions of the sera from newborn mice and the results determined on the following day (Fig. 1*a*). Test system: (1) antigen–serum of newborn mice; (2) antiserum–the monospecific serum of rabbits immunised with purified α-foetoprotein.

It is evident from Table 1 that in the serum, in the small and large intestine, liver, lung, brain, thymus, spleen and kidneys of adult mice there is a factor(s) suppressing AFP production in neonatal mice. Figure 1*b* and *c* show the AFP-suppressing effect of the extracts of small intestine. Extracts of 21-d-old mouse intestine produced a weaker effect than those of the adult. The factor is hardly detectable in small intestine of mice embryos, in the whole embryo or in striated muscles of adult mice. Heating of the small intestine extract at 60 °C for 45 min inactivated the suppression mechanism (data not shown). This high thermolability probably indicates the protein nature of the factor.

The function of embryonic antigens in tumour cells and the factors controlling their appearance remain obscure. Growth of tumours and transplants of foetal digestive tract is inhibited in newborn mice[4,5], and it may therefore be anticipated that suppression factor(s) controlling the synthesis of embryonic antigen are produced in the embryonic body, but are absent in

Table 1 AFP titre in the pooled sera of newborn mice inoculated with extracts of various tissues and killed on the day 10

Family number	Experimental Injections with 20% extract of:	Titre*	Control (without injection) —titre*
1	Adult small intestine	64	512
2	Adult small intestine	64	512
3	Adult small intestine	32	512
4	Adult small intestine	32	512
5	Adult large intestine	64	256
6	Adult large intestine	64	256
7	Adult large intestine	32	512
8	Adult large intestine	32	512
9	Adult kidneys	32	256
10	Adult kidneys	64	256
11	Adult liver	64	256
12	Adult liver	64	256
13	Adult lung	16	256
14	Adult lung	32	512
15	Adult spleen	64	256
16	Adult spleen	64	512
17	Adult thymus	64	512
18	Adult thymus	128	1024
19	Hanks' solution	256	256
20	Hanks' solution	256	256
21	Adult muscle	128	128†
22	Adult muscle	256	256†
23	Adult brain	64	256
24	Adult brain	64	256
25	Small intestine from 21-d-old mice	64	128
26	Small intestine from 21-d-old mice	64	256
27	Small intestine from 21-d-old mice	64	256
28	Small intestine from 21-d-old mice	64	256
29	Small intestine from embryos	256	256†
30	Small intestine from embryos	128	256
31	Embryos without gastrointestinal tract	512	512†
32	Embryos without gastrointestinal tract	512	512†
33	Adult serum	64	512
34	Adult serum	64	512
35	Hanks' solution	512	512
36	Hanks' solution	512	512

*Reciprocal to last dilution of serum of newborn mice in which AFP is determined in the microprecipitation test.

†Serum AFP titre of the experiment and control was the same, although the intensity of the precipitation line in the control was a little more distinct than in the experimental half.

The upper part (8 cm) of the small intestine was excised from adult BALB/c mice and its contents evacuated. It was then sectioned, washed repeatedly with Hanks' solution containing antibiotics (1000 U penicillin and streptomycin ml^{-1}), incubated in this solution for 30 min at 37 °C, and then washed repeatedly with Hanks' solution without antibiotics. The tissue was carefully ground in a mortar with glass powder; 20% extracts (20 g tissue 100 ml^{-1} Hanks' solution were prepared with 200 U ml^{-1} penicillin and streptomycin, and the homogenate centrifuged at 500 r.p.m. for 5 min. The supernatant was poured into plastic ampules and stored in liquid nitrogen (−196 °C) for 18 h, and subsequently at −22 °C. When required, ampules containing extracts were thawed and used for injections. Sterile extracts from liver, kidney, lung, brain, striated muscle and large intestine were prepared using this technique. Extracts were also prepared from small intestine of 21-d-old mice and mouse embryos taken at the end of the period of pregnancy. Extracts were also prepared from whole mouse embryos (head included) but with the gastrointestinal tract excised.

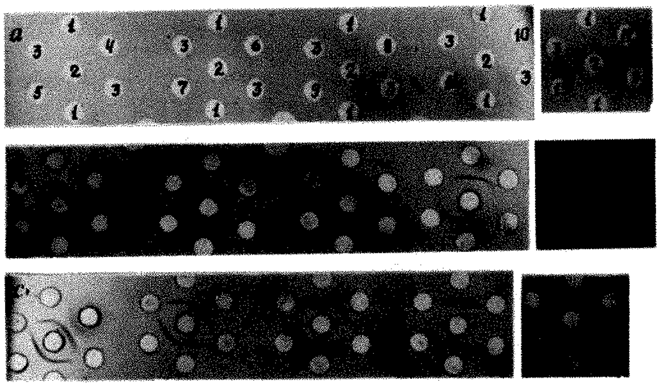

Fig. 1 *a*, Scheme of titration in the microprecipitation in agar. 1–2, test-system (1, antigen, 2, antiserum); 3, physiological solution; 4–13, twofold dilutions of the serum under study from 1:2 to 1:1024. *b*, Titration using serum from control animals (without inoculation of the extracts, day 10). Titre 1:256. *c*, Titration using serum from experimental animals inoculated with 20% extract from small intestine of adult mice (day 10). Titre 1:64.

adults. Using the suppression factor(s) from embryos or adults regulating induction and repression of antigens in normal embryonic cells, it might be possible to affect tumour cells synthesising the same embryonic antigens. These regulating factors were previously unknown, but one is possibly described here. Whether such a factor suppresses AFP synthesis in embryonic liver cells or the multiplication of the cells producing AFP, remains obscure. It seems possible that it would first normalise hepatoma cells, and then inhibit their multiplication. The detection of this suppressing factor seems to confirm the following general approach. Suppression factors affecting the synthesis of a given embryonic antigen must be determined in an organism by starting with that stage of ontogenesis in which their synthesis terminates, and sometimes after this stage. The systematic search for new hormones of embryonic and especially late embryonic and early postnatal periods would be particularly relevant.

We thank Dr F. J. Rauscher Jr for some research materials; Drs A. K. Jazova, A. I. Gousev and A. R. Karamova for the test system; and Drs H. I. Abelev and L. N. Fontalin for discussions.

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Evidence against clustering of thymine dimers in ultraviolet-irradiated DNA

IRRADIATION of DNA with ultraviolet light causes chemical and physical alterations. From the chemical point of view the main effect of 254 nm UV light is the formation of pyrimidine dimers. The physical consequence of this is a destabilisation of the DNA double helix, presumably by formation of small denatured regions (defects).

According to Shafranovskaya *et al.*[1], the number of defects as determined by the kinetic formaldehyde method is approximately equal to the number of thymine dimers at low fluences of ultraviolet light, but increases much less rapidly than the yield of dimers at fluences above 10 J m^{-2}. To explain these results they assume that at low fluences every dimer produces a defect, but that at higher fluences dimers are preferentially formed at pre-existing defects after migration of the excitation energy over large distances, up to several thousands of base pairs.

To check this important hypothesis we repeated the experiments of Shafranovskaya *et al.*[1] very carefully taking every precaution to prevent artefacts. Contrary to their findings the concentration of defects did not depart significantly from a linear dependence on ultraviolet fluence. Our experiments therefore contradict the assumption of large distance energy transfer in DNA but of course do not exclude the possibility of energy migration over short distances.

The theory of the kinetic formaldehyde method[2,3] predicts that denaturation of DNA by the action of formaldehyde follows the equation

$$(-\ln\theta)/t = p\nu + 2vc + pvt \qquad (1)$$

in which c is the initial number of defects per base pair, v the rate of reaction of formaldehyde with base pairs at the boundary of a defect, p the rate for reaction with base pairs in a stable helix and ν the average number of base pairs opened up together in the latter process. Under suitably chosen conditions the term $p\nu$ can be neglected. The degree of helicity θ is defined as

$$\theta = (A_f - A)/(A_f - A_i),$$

where A_i, A and A_f are the initial absorbance, the absorbance at time t and the final absorbance, respectively.

Plotting $(-\ln\theta)/t$ against t gives a straight line with a slope pv and an intercept $2cv$ on extrapolation to $t=0$, and c can be calculated if v is known.

The quotient $(-\ln\theta)/t$ is rather sensitive to errors in A_i. Because the absorbance at the time of mixing of DNA and

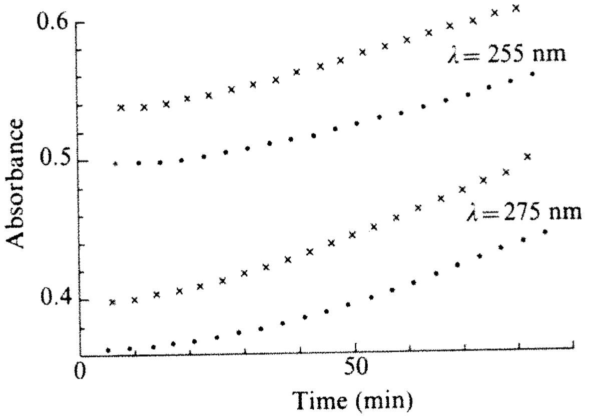

Fig. 1 Change in absorbance of DNA under the action of formaldehyde: ●, non-irradiated DNA; ×, DNA irradiated with 66 J m^2 (see legend to Fig. 3). Solvent for DNA was 0.01 M borate buffer, *p*H 8.7, containing 0.019 M NaCl and 5×10^{-4} M EDTA (ref. 3). Formaldehyde was prepared from paraformaldehyde[5], kept at 100 °C for 5–10 min[6], neutralised and taken up in concentrated buffer solution to obtain a concentration of 6.7% (w/v) in the same solvent as used for DNA. The final solution was kept at room temperature for at least 2 d to eliminate undesirable drifts in absorbance. Equal weights of DNA and formaldehyde solution were brought into both halves of a two-compartment spectrophotometer cell, equilibrated at 47.5 °C for 60 min and then mixed. During the mixing and subsequent measurements the temperature in the cell remained constant within 0.02 °C. The transmission of the reaction mixture was measured as a function of time in a Zeiss PMQ II spectrophotometer connected with a digital voltmeter to improve the sensitivity. At 90 min after mixing the temperature of the cell holder was increased to 70 °C for 10 min to complete the reaction and then brought down again to determine the final transmission.

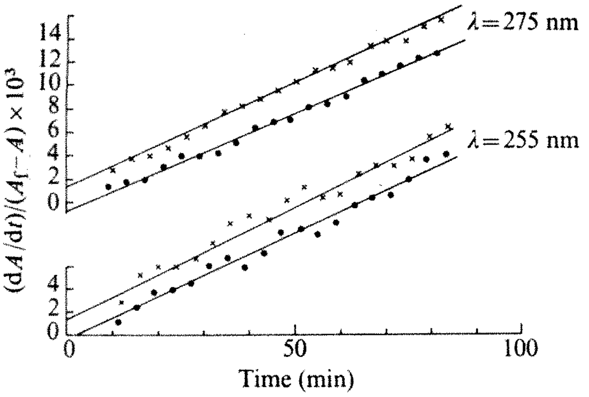

Fig. 2 The kinetics of the reaction of formaldehyde with T2-DNA at 47.5 °C presented as a plot of $(dA/dt)/(A_f-A)$ against t at wavelengths of 255 and 275 nm. ●, non-irradiated DNA; ×, DNA irradiated with 66 J m^{-2}.

formaldehyde cannot be determined very accurately we analysed our data by computer using the equation

$$(dA/dt)/(A_f-A)=2vc+2pvt \tag{2}$$

which is in fact the differential equation on which equation (1) is based. The relevant quantity $2vc$ is obtained in the same way as with equation (1), that is, by determining the intercept of a straight line at $t=0$.

The experiments were performed with DNA from the same bacteriophage, T2, as that used by Shafranovskaya *et al.*[1]. In this DNA the only pyrimidine dimers produced are thymine dimers. Figure 1 shows the change in absorbance of DNA caused by the action of formaldehyde. All measurements were carried out at 255 and 275 nm, the first wavelength being that of the isosbestic point for the reaction between formaldehyde and denatured DNA. Here the absorbance change is only caused by DNA denaturation, whereas the (larger) change at 275 nm contains a contribution from the formaldehyde reaction itself.

Examples of plots according to equation (2) are given in Fig. 2 for non-irradiated DNA and DNA irradiated with 66 J m^{-2}. The fact that the slopes for both wavelengths do not differ significantly confirms the expectation that reaction with formaldehyde goes hand in hand with denaturation. The wavelength of measurement is therefore not very critical. For reasons as yet unknown, the value of $2vc$ for non-irradiated DNA is slightly negative.

Figure 3 shows that the concentration of defects in DNA increases in proportion to ultraviolet fluences from 0 to 165 J m^{-2}.

These results together with their standard errors are summarised in Table 1. The figures obtained from plots of $(-\ln\theta)/t$ (using extrapolated values of A_i) are also given. Although they are somewhat less reliable, they seem to lead to the same conclusion.

We are unable to indicate exactly why our experiments show a linear dependence of $2vc$ on ultraviolet fluence, whereas those of Shafranovskaya *et al.* do not. It should be stressed, however, that without careful precautions erroneous and irreproducible results may be obtained because the change in absorbance after mixing may be caused partly by factors other than the reaction of DNA with formaldehyde. First, the temperature must be kept constant within narrow limits. Second, the absorbance of formaldehyde shows a slow drift after a change of temperature, the effect of which is largely eliminated by special pre-equilibration procedures (Fig. 1). Third, it seems that without the pre-equilibration procedures the absorbance of formaldehyde showed a slow drift after mixing with an equal volume of buffer. Fourth, imperfectly cleaned cells sometimes showed a slowly varying blank absorbance.

To check whether the dose–effect curve is linear one does not need to know the value of v. This value was obtained by Shafranovskaya *et al.* by calibrating the kinetic formaldehyde method using single- or double-strand breaks in DNA as defects, the number of which can be determined by ultracentrifugation. Rahn and Stafford[4] produced evidence that for dimer defects the value of v is much smaller than for break defects. This may explain why at high doses the number of defects seems to be much smaller than the number of dimers, determined by chromatographic techniques. The evidence of Rahn and

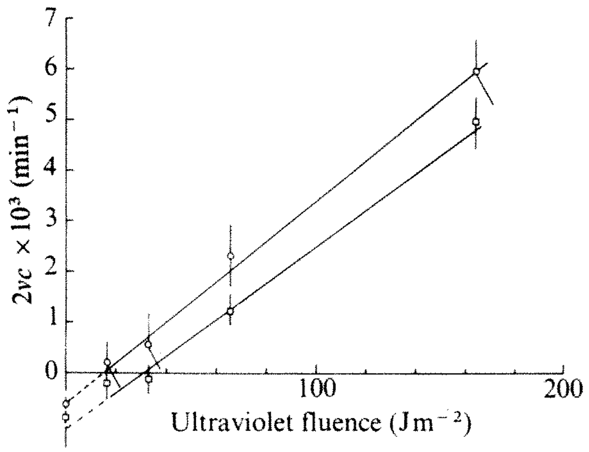

Fig. 3 Relative concentration of defects (value of $2vc$) in T2-DNA as a function of ultraviolet fluence: ○, measurements at 255 nm; ☐, measurements at 275 nm. Irradiations were carried out with a 15 W low pressure mercury lamp. Solutions (absorbance 0.4) were irradiated horizontally in silica spectrophotometer cells with a light path of 1 cm and containing a small magnetic stirrer. The lamp output was monitored with a Yellow Springs Instrument–Kettering radiometer. The intensity at the position of the cell was 0.65 J m^{-2} s^{-1} and was derived from the inactivation rate of T4 and øX174 phage assuming 37% survival at 2.83 and 8.07 J m^{-2}, respectively[7,8]. The experimental points for irradiated DNA are the weighted averages of two or three measurements. The straight lines were obtained by least squares analysis of all these points. Each of the two points for non-irradiated DNA is the weighted average of seven measurements. Bars represent s.e.

Table 1 Analysis of the measurements*

Calculation number	Wavelength (nm)	$2vc_0\times10^3$ (min^{-1})	Irradiated DNA Intercept × 10^3 (min^{-1})	Slope × 10^3 (min^{-1}J^{-1}m^2)
A	275	−0.9±0.1	−1.1±0.2	0.036±0.003
	255	−0.7±0.2	−0.5±0.2	0.040±0.003
B	275	−0.5±0.2	−0.5±0.9	0.03±0.02
	255	−0.1±0.2	−0.1±1.2	0.03±0.02

*Kinetic curves were analysed using one of the formulae (1) and (2) from the text. The data under A were obtained using equation (2), the data under B using equation (1). From the seven measurements for non-irradiated DNA, a weighted average $2vc_0$ was calculated (c_0: the concentration of defects at zero UV fluence). The values of $2vc$ at ultraviolet fluences between 16 and 170 J m^{-2} were fitted by a straight line (Fig. 3); the intercept and the slope of this line are given. The intercept does not differ significantly from $2vc_0$.

Stafford together with our linear dose–effect relationship makes it very unlikely that the hypothesis of clustering of thymine dimers through long range energy migration is correct.

We thank Messrs J. B. Verberne and J. W. Niemantsverdriet for assistance.

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Received February 17; accepted March 26, 1975.

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Assembly of DNA and protein during replication in HeLa cells

REPLICATION in eukaryotic cells includes not only DNA synthetic processes[1-4], but also the temporally coordinate[5-7] and non-coordinate[8,9,21] synthesis of chromatin proteins, as well as assembly of these constituents into interphase chromosomes. While much is known about DNA, histone and non-histone chromatin protein biosynthesis, less is known about the time, place and manner of association of proteins with DNA. This communication presents evidence that (1) DNA is replicated with pre-existent chromatin protein still attached; (2) chromatin proteins protect DNA from nuclease digestion in a characteristic manner, unchanged by replicative processes, and (3) addition of new chromatin protein to nascent DNA occurs after a measurable lag period.

Nuclease digestion was used as a probe to examine the extent of DNA-protein associations. Two nucleases are well characterised in chromatin degradations: (i) pancreatic DNase I digests chromatin in a biphasic manner in which DNA is quantitatively degraded, the reaction rate being dependent on the enzyme-substrate ratio[10,11]; (ii) staphylococcal nuclease ceases digestion after hydrolysis of 45–50% of chromatin DNA (limit digest) irrespective of enzyme load[11,12,16,22]. Since the two nucleases behave so differently with chromatin, I used each enzyme in parallel digestion studies to ensure that the data were qualitatively in agreement and were not produced by anomalous interplay of either enzyme with the complex deoxyribonucleoprotein substrate.

To study the timing of addition of protein to chromatin, newly synthesised DNA was assayed for nuclease susceptibility. Cells were incubated with ^{3}H-thymidine for periods of 30 s to 32 min, or 12 h for controls (Fig. 1). The kinetics of DNase I digestion of nuclei prepared from each incubation are given in Fig. 1. The most briefly labelled DNAs (30 s–1 min ^{3}H-thymidine) were substantially more accessible to nuclease attack than control chromatin, being intermediate between values for free DNA (>95% degradation) and control chromatin. This enhanced susceptibility was of brief duration, for resistance to digestion increased with the duration of the labelling period, finally to the level of control chromatin within 10-20 min. In Fig. 1*b* the results are plotted as the amount of each sample digested in 45 min as a function of labelling period, to illustrate more clearly the kinetics of maturation of nuclease resistance. These results are qualitatively the same with both DNase I and staphylococcal nuclease.

That the progression of nuclease-susceptible nascent DNA

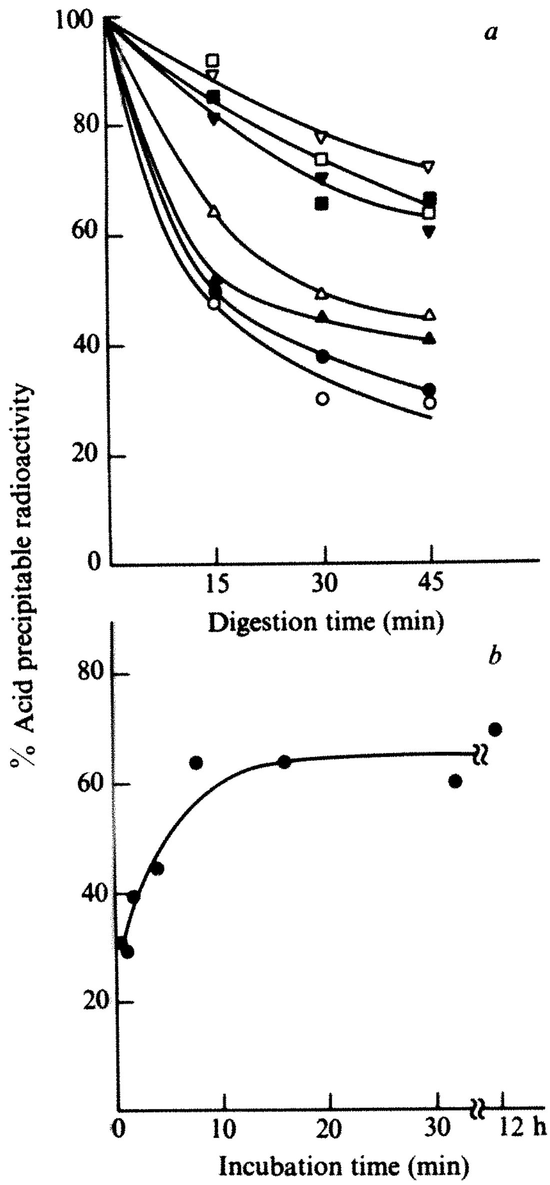
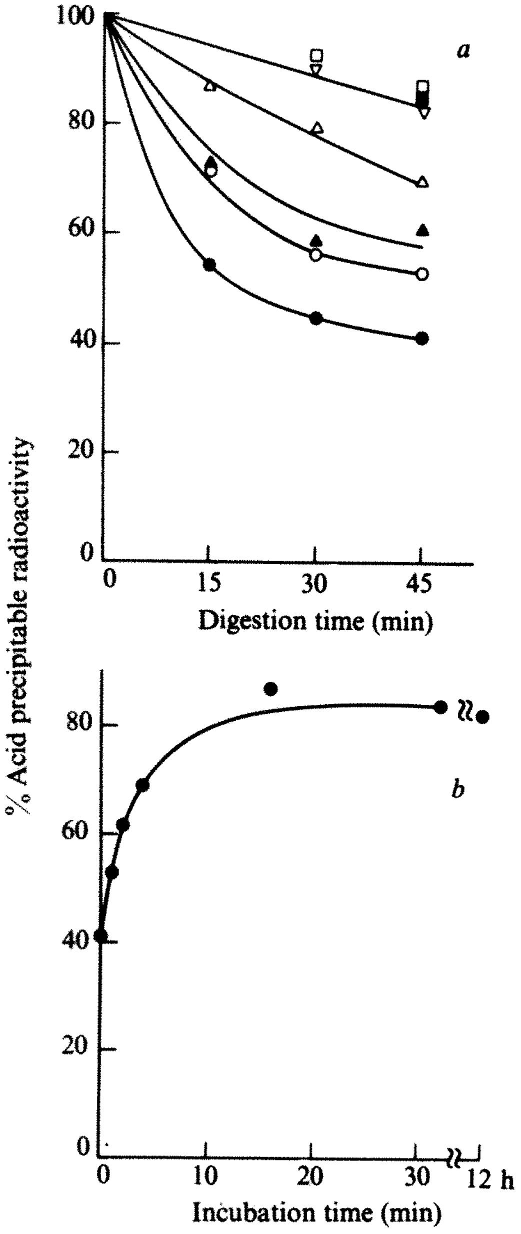


Fig. 1 *a*, Kinetics of DNase I digestion of nuclei labelled for 0.5 (●), 1 (○), 2(▲), 4 (△), 16 (□), and 32 min (■), and for 12 h (▽) with thymidine. *b*, Nuclease resistance of labelled chromatins after digestion for 45 min. Note different abcissas in *a* and *b*. HeLa S-3 cells, maintained at $2–5\times10^5$ ml^{-1} in Eagle's MEM containing 5% horse serum were concentrated to 2×10^6 ml^{-1} for labelling. Samples (20 ml) of concentrated cells were incubated with 200, 200, 150, 100, 50, 25 and 10 µCi ^{3}H-thymidine (6.7 Ci mmol^{-1}) for 0.5, 1, 2, 4, 8, 16 and 32 min respectively. Control cells were labelled for 12 h at 0.01 µCi ml^{-1}. Labelling was terminated by immersion of the tube in ice and addition of 2 volumes of RSB (10 mM Tris-HCl, *p*H 7.6, 1.5 mM $MgCl_2$, 10 mM NaCl) containing 0.2% sodium azide. The cells were collected by centrifugation at 1,800*g* (maximum) for 1 min. The pellet was resuspended and washed again in 20 ml RSB. After resuspension in RSB the cells were swollen for 10 min and then 0·1 volume 10% Triton X-100 was added. The cells were swirled on a Vortex mixer for 30 s and then centrifuged at 1,800*g* for 1 min. The nuclei were washed twice more in RSB. The final nuclear suspension in RSB was adjusted to a concentration of 3.0 A_{260} units ml^{-1}, as measured in 1% sodium dodecyl sulphate. Samples (0.1 ml) of the nuclear suspension were added to tubes, followed by 0.8 ml RSB. Then, 5 µg DNase I (Worthington) in 0.1 ml RSB was added and, after mixing, the tubes were placed in a 37 °C water bath. At the indicated times, reactions were stopped by placing tubes in ice and adding 2 ml 10% trichloracetic acid. Samples (100 µg) each of bovine serum albumin and calf thymus DNA were added to each tube and the precipitates were collected on Whatman GF/C filters. To each filter, 0.2 ml of a 9:1 NCS (Amersham/Searle)–water mixture was added, followed by 10 ml of 0.4% PPO in toulene. Vials were incubated for 12-16 h at 37 °C before counting in a Beckman LS-250 liquid scintillation spectrometer.

to the relatively nuclease-resistant control chromatin represented a true precursor–product relationship, was demonstrated by labelling nascent DNA by brief incubation with isotope, and following the fate of the incorporated radioactivity after removal of isotope (Fig. 2). Cells were incubated with ^{3}H-thymidine for 90 s and, after removal of isotope, samples were collected at intervals for 30 min. The nuclease resistance of the 90 s labelled material was again 50% that of controls. Resistance of the radioactive DNA to digestion increased progressively with time to reach controls after 10–15 min of further incubation. That the digestibility of the first sample taken after labelling was already less than the 90 s sample, reflects the 5 min required for the washing procedure. There was no evidence that newly synthesised DNA was protected from nucleases, relative to controls, in contrast to a report on nuclease susceptibility of nascent DNA in chick erythrocyte chromatin[15]. The time for unidirectional synthesis of replicon-sized units (3.5–15 μm)[1] of DNA at chain elongation rates of 0.8–2 μm min^{-1} (refs 1, 3 and 18–20) is about 5–15 min. Thus the period of 10–15 min observed for maturation of nuclease resistance, interpreted to represent chromatin protein addition, corresponds with that for completion of replicons.

Fig. 2 Digestibility of briefly labelled chromatin DNA after removal of label. Cells, as described in the legend to Fig. 1, were incubated with 10 μCi ^{3}H-thymidine ml^{-1} for 90 s. To terminate labelling, non-radioactive thymidine was added to 50 μg ml^{-1}. A sample was removed for the initial sample and the cells were centrifuged at 1,800*g* (maximum) for 1 min. Cells were resuspended in 37 °C medium containing 50 μg thymidine ml^{-1}, centrifuged again, and finally resuspended in fresh thymidine-containing medium for the times indicated. Samples were collected and processed at the times indicated as described for Fig. 1. ●, ^{3}H-thymidine incubation for 90 s; ○, zero time after isotope removal; ▲, further incubation for 2 min; △, 4 min; ■, 16 min; □, 32 min; ▽, one generation label.



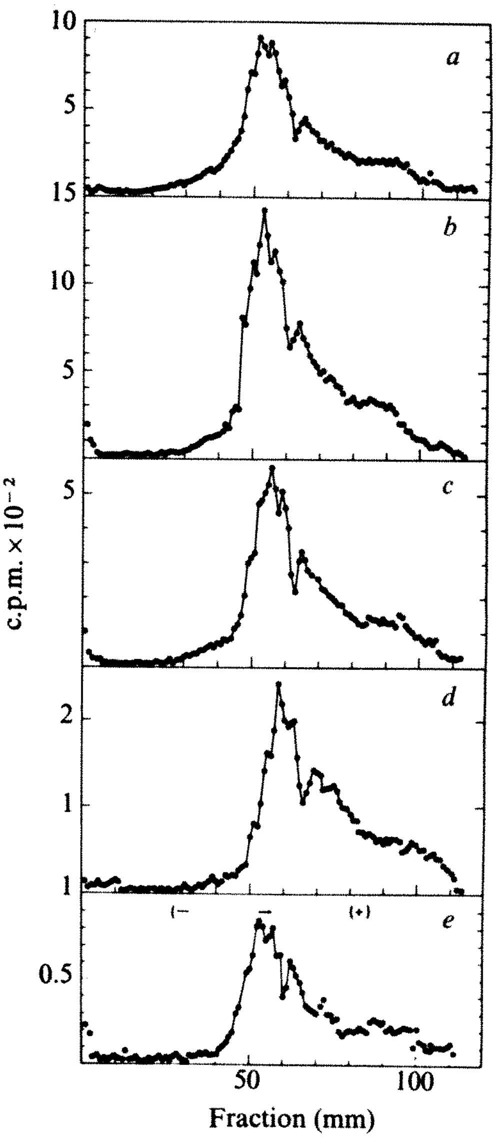


Fig. 3 Polyacrylamide gel electrophoresis of nuclease-resistant DNA fragments from staphylococcal nuclease limit digests. *a*, one generation label; *b*, 10 min; *c*, 4 min; *d*, 90 s; *e*, 45 s. Cells were incubated with 10 μCi ml^{-1} (*b*–*e*) or for 16 h with 0.01 μCi ml^{-1} ^{3}H-thymidine (*a*), respectively. Nuclear digestions were performed in 5 mM phosphate *p*H, 2.5×10^{-5} M Ca^{2+} with 1 μg of staphylococcal nuclease. After 90 min precipitates were collected by centrifugation at 17,000*g* for 10 min. The precipitates were dissolved in 1% SDS, 0.1 M NaCl, 1 mM EDTA, 10 mM Tris, *p*H 7.6, extracted three times with chloroform isoamyl alcohol (24:1), and precipitated with 2 volumes ethanol at –20 °C for 16 h. DNA was dissolved in electrophoresis buffer containing 6 M urea. The samples were applied to 0.6 × 10-cm 6% acrylamide gels (20:1 acrylamide-*bis* acrylamide) and were subjected to electrophoresis in buffer containing 0.089 mM Tris, *p*H 7.6, 0.90 M boric acid and 6.5 mM disodium EDTA [17] for 2 h at 2 mA per gel. Gels were scanned at 260 nm in a Beckman Acta III recording spectrophotometer equipped with a linear transport device. The gels were then fractionated into 1-mm segments with a Mickle Gel Slicer (Brinkmann Instruments). Gel slices were incubated in 0.5 ml NCS (Amersham/Searle)–water (9:1) at 50 °C, 12 h. Scintillation fluid was then added, and after 6 h at 37 °C counting was carried out as in Fig. 1.

Staphylococcal nuclease digestions produce a true limit digest of chromatin[11,21,22] and the resistant DNA can be resolved in polyacrylamide gels as a series of fragments of discrete sizes[16]. By this method, the size classes of resistant DNA from control chromatin were compared with patterns from briefly labelled chromatins as described in Figs 1 and 2 (Fig. 3). The patterns of nuclease-resistant DNA fragments derived from cells labelled for 45 s, 90 s, 2 min, 4 min, 10 min and 17 h were all the same. Recoveries from gels averaged 80-85% in all cases.

These results can be interpreted in the form of a simplified model. Chromatin is represented as DNA complexed with discrete protein oligomers[13,14], possibly of different, but a limited variety of sizes. As DNA is replicated, these protein oligomers segregate with the daughter chromosomes such that the newly synthesised DNA has half the protein content of mature chromatin. As replicon-sized DNA fragments are synthesised (about 10-20 min), new protein is added and nuclease resistance increases to that of parental chromatin. Because the size of nuclease-resistant DNA fragments is identical at all times, I propose that chromatin proteins (histones) associate with the new DNA in oligomeric form. Thus, all chromatin, regardless of the state of maturation and nuclease resistance, yields the same size classes of resistant DNA fragments.

I thank Dr R. Simpson, in whose laboratory this work was carried out, for advice and encouragemet. The late Dr H. A. Sober gave continuous interest and support.

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Received January 6; revised March 31, 1975.

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Denaturation of polyoma DNA by phage T4 gene 32 protein

POLYOMA virus is a small oncogenic virus containing a circular DNA, the identification and location of defined regions of which provides physical reference points which are important for correlation in genetic and biochemical studies. The protein coded by gene 32 of bacteriophage T4 binds tightly to single-stranded DNA and RNA and facilitates denaturation and renaturation of double-stranded DNA[1,2]. Using glutaraldehyde fixation gene 32 protein denaturation of double-stranded DNA can be observed using electron microscopy at physiological temperatures, and denaturation maps are produced which closely resemble those obtained with heat and alkaline denaturation[2]. Use of gene 32 protein in binding experiments with circular supercoiled DNA molecules from polyoma virus and simian virus 40 (SV40) has already shown that only one denaturation loop is formed per molecule[2-4] reflecting possible structural constraints in the DNA. The characterisation and mapping of the preferred denaturation site(s)[4] is also of interest since these putative A+T-rich region(s) are likely to be related to the binding sites of enzymes using DNA as a template. In the present study the use of two different specific restriction enzymes (endonucleases) has enabled us to identify five regions of polyoma DNA which are preferentially denatured by phage T4 gene 32 protein, and the determination of their precise locations on the circular viral DNA.

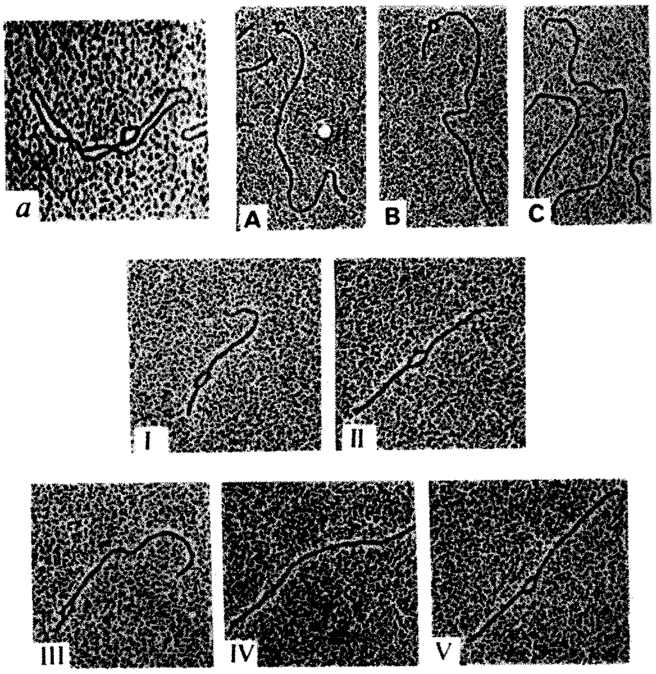


Fig. 1 Electron micrographs of polyoma DNA molecules carrying a denaturation loop induced by gene 32 protein. *a*, Closed polyoma DNA molecule carrying a denaturation loop. A, B and C, *Eco*RI cleaved polyoma DNA molecules carrying denaturation loop induced by gene 32 protein at three different sites. I and II, 43% fragment from *Hin*III digestion of Py DNA denatured by gene 32 protein with a denaturation loop at one of two sites. III-V, 57% fragment from *Hin*III digestion of polyoma DNA denatured by gene 32 protein with a denaturation loop at one of three sites. Gene 32 protein was purified from *E. coli* cells infected with an amber mutant of T4 bacteriophage (lysozyme⁻) obtained from the New England Enzyme Centre, Massachusetts, using the method of Alberts and Frey[1]. Polyoma DNA was labelled with ^{3}H-thymidine and extracted from cells infected with *A-2* strain of large plaque virus at low multiplicities of infection[5] by the method of Hirt[6] followed by phenol extraction, equilibrium centrifugation in CsCl–ethidium bromide, and purification by neutral sucrose density gradient centrifugation. Complexes of polyoma DNA (form I) with T4 gene 32 protein were prepared according to Delius *et al.*[2]. After fixation in glutaraldehyde the sample was dialysed against 0.01 M Tris-HCl, *p*H 7.4, containing 0.01 M NaCl and 1 mM EDTA at 4 °C, and concentrated. Conditions were then adjusted and digestion carried out with endonuclease *Eco*RI and *Hin*III as described by Griffin *et al.*[7]. The preparation of samples for electron microscopy was carried out as described by Davis *et al.*[8]. An internal length standard of PM2 DNA (taken as 9,900 nucleotide pairs) was used. Measurements were made using a Hewlett Packard digitiser interfaced with a 9820A calculator.

When polyoma DNA was incubated with gene 32 protein (Fig. 1*a*), only one denaturation region was seen per molecule of DNA (with one exception) and the size of the denaturation loop formed was about 5% (or 260 base pairs) of the full length of the molecule. The presence of gene 32 protein increased the length of the DNA molecule by about 4%.

When circular polyoma DNA molecules containing a

Table 1 Possible locations and frequencies of the individual denaturation sites induced by gene 32, measured on the two fragments produced by digestion of polyoma DNA using endonuclease *Hin*III

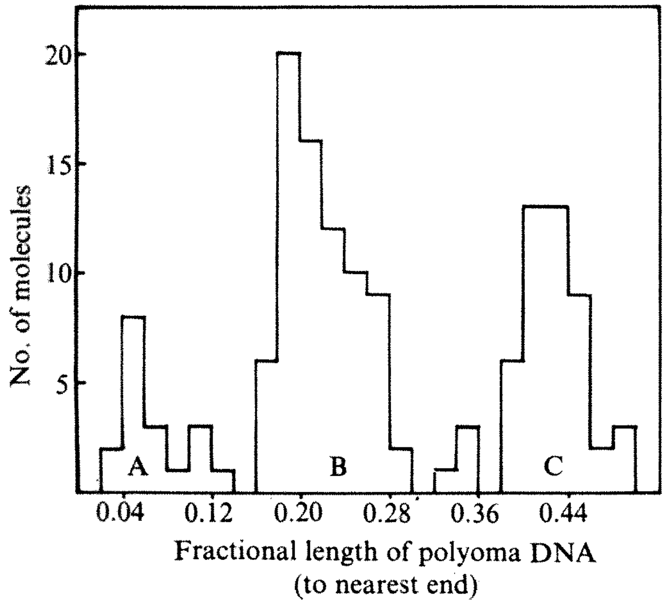
*Hin*III fragment, gene 32 protein complex	Possible locations	s.d.	Frequency
I	0.08* or 0.36	± .03	11%
II	0.20* or 0.24*	± .02	33%
III	0.03 or 0.47*	± .02	14%
IV	0.12 or 0.44*	± .02	27%
V	0.20* or 0.36	± .01	14%

Measurements on *Hin*III fragments were converted to percentage total genome, referred to the *Eco*RI cleavage site and mapped to the nearest end of a full length linear molecule. The two possible locations for each denaturation loop, depending on the orientation of the fragment, are shown with the most probable location asterisked. I and II correspond to 43% *Hin*III fragment species (Fig. 1); III, IV, and V correspond to 57% *Hin*III fragment species (Fig. 1).

denaturation loop were digested with restriction enzyme *Eco*RI (which cuts polyoma DNA at one unique site[7,9]) three types of molecule were observed, each with the denaturation loop at a particular site (Fig. 1A, B and C). Figure 2 shows that the three preferred sites for denaturation are located, respectively, at 0.07, 0.23 and 0.42 fractional length from the nearest end of the molecule and with frequencies of 12%, 52% and 36%. These observations are in agreement with those reported by Yaniv *et al.*[4].

The unambiguous location of these sites on the circular DNA molecule, however, could only be achieved using another specific restriction enzyme capable of distinguishing between the two ends of the linear molecule cleaved by *Eco*RI. This was accomplished using endonuclease *Hin*III which cuts polyoma DNA at two sites[7] generating two fragments, one 57±2% and the other 43±2% of the genome (Fig. 3). When polyoma DNA gene 32 protein complexes were digested using this enzyme, the two fragments were obtained, one about 43% of the genome with a denaturation loop at one of two sites, and the other about 57% of the genome with a denaturation loop at one of three sites (Fig. 1, I–V). Table 1 shows that four of the sites found on the *Hin*III fragments must be located in positions equidistant from the *Eco*RI site and thus together comprise two of the sites found on the *Eco*RI linears. As we knew the location of the two fragments on the circular map[7], we determined the absolute positions of the loops by comparing the *Hin*III data with the distribution of loops on the *Eco*RI linears.

Fig. 2 Location of denaturation sites (A, B, C) induced by gene 32 protein on *Eco*RI cleaved polyoma DNA gene 32 complexes (expressed as fractional length to the nearest end). Measurements were carried out to the midpoint of the denaturation loop. Average locations were: A, 0.07 ± 0.03; B, 0.23 ± 0.06; C, 0.42 ± 0.07. Relatives frequencies were: A, 12%; B, 52%; C, 36%.



Taking the 43% fragment first, each of the sites has two possible map locations, depending on which way the fragment is oriented (Fig. 4*b* and *c*). Because one of the loops is located at about the midpoint of the fragment either at 0.20 or 0.24 on the map, however, this immediately fixes the position of this denaturation region (II in Figs 1 and 4) as corresponding to B at 0.23 from the *Eco*RI linear map (also shown for comparison in Fig. 4). The other denaturation region observed on the 43% fragment could occupy either position 0.08 or 0.36 (Fig. 4*b*). Comparison with the *Eco*RI linear map shows that there is a denaturation region which could correspond to the 0.08 position but none at 0.36. This observation identifies site I (Figs 1 and 4) as being A on the *Eco*RI map. On comparing the frequencies of these sites (I and II) with the *Eco*RI frequencies, we found that whereas the frequency of denaturation site I on the small fragments agrees with that observed for site A on the *Eco*RI linears (11% as opposed to 12%) site II only accounts for 33% of the denaturation sites observed (as opposed to 52% incidence of denaturation site B on *Eco*RI). This would therefore imply that site B is a 'double' site, that is, one made up of two sites, equidistant from the *Eco*RI cleavage site. This interpretation would predict that another denaturation loop would be found on the 57% fragment, corresponding to map position about 0.77 (Fig. 3); site V fits these requirements (Fig. 4*g*). Its frequency of 14% brings the overall frequency of II+V to 47% in reasonable agreement with the 52% frequency observed for the B loop on the *Eco*RI linears. The alter-

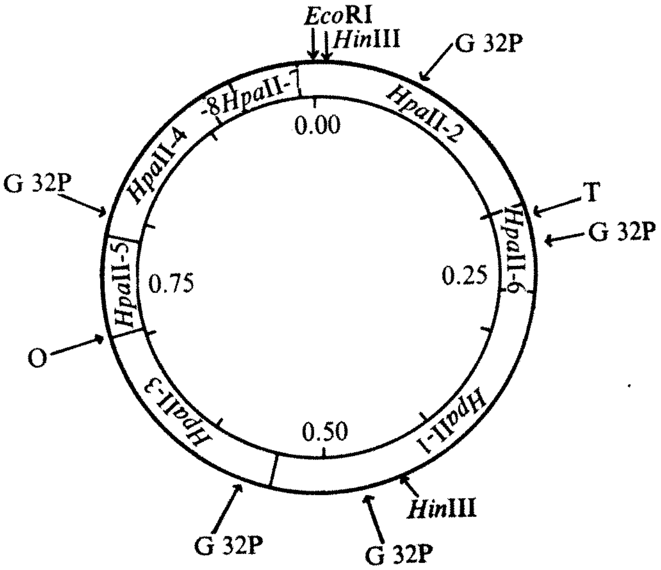
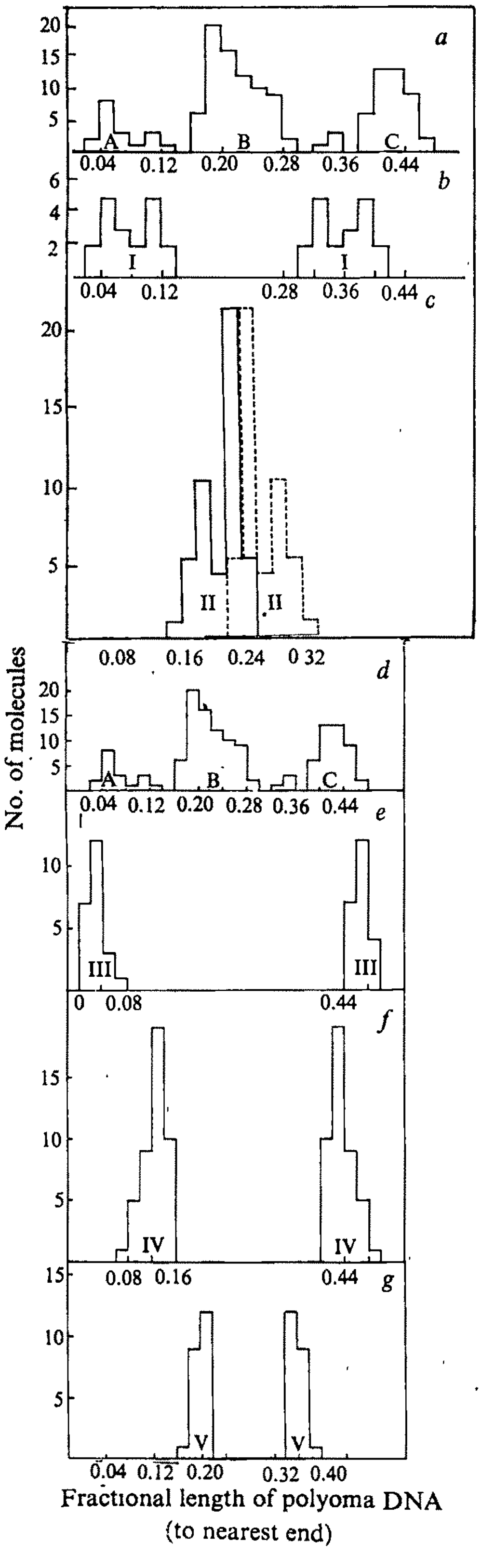


Fig. 3 Polyoma DNA map showing cleavage sites for enzymes *Eco*RI, *Hin*III and *Hpa*II (from Griffin *et al.*[7]), and gene 32 protein (G32P) denaturation regions referred to in the text. O and T are origin and termination of DNA synthesis respectively. Full length is 1 unit.

Table 2 Frequencies and locations of the various denaturation sites measured on *Hin* III fragments compared with distribution on *Eco*RI linears

*Eco*RI		*Hin*III	
Location	Frequency	Location	Frequency
0.07 ± .03	12%	0.08 ± .03	11%
0.23 ± .06	52%	0.23 ± .03	47%
0.42 ± .07	36%	0.44 ± .04	41%

Fig. 4 Alternative map locations of denaturation sites induced by gene 32 protein on the *Hin*III fragments expressed as the fractional genome length from the nearest *Eco*RI end. *a–c*, 43% *Hin*III fragment. *a*, Distribution of denaturation loops on *Eco*RIcleaved polyoma DNA–gene 32 protein complexes for comparison. *b*, *Hin*III 43% fragment with denaturation loop (I) shown in Fig. 1. *c*, *Hin*III 43% fragment with denaturation loop (II) shown in Fig. 1. *d–g*, 57% *Hin*III fragment. *d*, Distribution of denaturation loops on *Eco*RI linears as in (*a*) for comparison. *e*, *Hin*III 57% fragment carrying denaturation loop (III) as shown in Fig. 1. Note that the histogram on the right is not the 'mirror image' of the one on the left, as is the case in (*f*) and (*g*). This is a result of the second possible location extending to either side of the midpoint (0.5), and being expressed as fractional genome length from the nearest *Eco*RI end. *f*, *Hin*III 57% fragment carrying denaturation loop (IV) as shown in Fig. 1. *g*, *Hin*III 57% fragment carrying denaturation loop (V) as shown in Fig. 1.



native position for denaturation site V at 0.36 does not correspond to a site found on the *Eco*RI map.

For the two other denaturation sites observed on the large *Hin*III *fragment* (57%) the same approach was followed. Figure 4*e* and *f* shows that sites III and IV must occupy approximately equidistant positions in relation to the *Eco*RI cleavage site: one at map position 0.47 and the other at position 0.56 together accounting for site C at 0.43 on the *Eco*RI map (Fig. 4*d*). When the frequencies observed for these two locations are added a total of 41% is obtained in reasonable agreement with that observed for C (36%). Finally, data (not shown) from digestion of polyoma DNA gene 32 protein complexes with another enzyme—*Hpa*II—generating eight fragments[7], also confirms the presence of five easily denaturable regions.

In summary, there seems to be on polyoma DNA five sites which are readily denatured by gene 32 protein with map positions (Fig. 3) of approximately 0.08, 0.23, 0.47, 0.56 and 0.80 and frequencies of 11%, 33%, 14%, 27% and 14%, respectively. The *Hin*III data when expressed as percentage total genome and referred to the nearest end compares well with the *Eco*RI linear map (Table 2). Note that the preferred sites for denaturation by gene 32 protein correspond to *Hpa*II fragments 2, 6, 1, 3 and 4 (Fig. 3) and that 2 and 6 were the fragments found by Giffin *et al.*[7] as having the highest A+T/G+C ratio. The origin of DNA replication which has been mapped at 0.71 (refs 7 and 10) does not seem to be associated with an A+T-rich region whereas termination of DNA synthesis at 0.21 (Fig. 3) is closely associated with the most frequent denaturation site at 0.23. The present work using two different restriction enzymes overcomes the previous difficulties in trying to locate A+T-rich regions in circular viral DNA molecules[11,12]. The functional significance of each of the A+T-rich regions described in this study remains to be established.

We thank Dr M. Fried for laboratory facilities, for encouragement and for discussions; Dr L. V. Crawford (in whose laboratory the work was initiated) for help in preparing the manuscript; Dr B. Griffin for help with some experimental details; and Miss F. Crew for assistance. We acknowledge gifts of purified polyoma DNA from Dr M. Fried, of *Eco*RI from Dr R. Kamen and *Hin*III from Dr R. Roberts.

Note added in proof: Lescure and Yaniv (personal communication) also have evidence suggesting the presence of a gene 32 protein induced denaturation site mapping at ~0.80.

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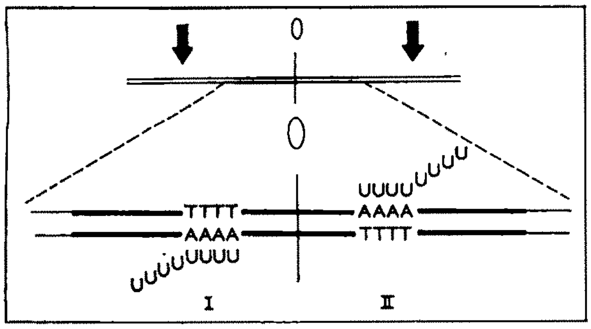
Proximity of deoxyadenylic-thymidylic acid clusters to origin of DNA replication in *E. coli* 15T$^-$ cells

CLUSTERS of deoxyadenylic-thymidylic (dA-T) and deoxycytidylic-deoxyguanylic acid (dC-dG) residues have been detected in DNAs from many sources[1,2]. The purine or pyrimidine clusters in denatured DNA can hybridise with complementary polyribonucleotides and the greater density of the complexes means that they can be separated from unreacted DNA by isopycnic centrifugation in CsCl or $\mathrm{Cs_2SO_4}$ (refs 1 and 2). These clusters are estimated to be 10–100 residues long and are distributed throughout DNA at intervals of 10^5–10^6 daltons (ref. 3). The dC-dG clusters have been implicated in the regulation of transcription in viruses[4–8], bacteria[1,9] and in nuclei (P. K. Bhattacharya and H.K., unpublished) from eukaryotic cells (for reviews see refs 3 and 10). Less is known about the function of clusters of dA–T residues which are as common in most DNAs[1]. Although in adenoviruses the presence of dA-T clusters has been correlated with the oncogenic potential[11] and Borst and Ruttenberg[12] found runs of dT on the transcribed strand of mitochondrial DNA, no further efforts have been made to associate regulatory or other functions with the presence of these clusters. We now report observations which suggest that clusters of dA-T may be involved in the initiation of DNA synthesis. The rationale for our investigation was that if such clusters were detected at or near the replication origin, it would be consistent with the proposition that they function in the initiation of DNA replication.

If a synchronised culture of *Escherichia coli* is pulsed with ^{3}H-thymidine when DNA replication resumes and subsequently chased with cold thymidine, newly replicated DNA near the origin is labelled whereas that farther from the origin and all of the unreplicated portion of the DNA is unlabelled (Fig. 1). Since replication in *E. coli* is bidirectional[13,14], labelled regions on both sides of the origin (0) would be anticipated (thick lines).

Fig. 1 Schematic representation of the experimental design. O, point of origin (starting point of DNA replication); I and II, two dA-T clusters of opposite polarity; the thick and the thin lines denote the labelled and unlabelled portions of cellular DNA, respectively; strings of Us, poly(U); the two arrows show the positions of breaks in DNA during shearing.



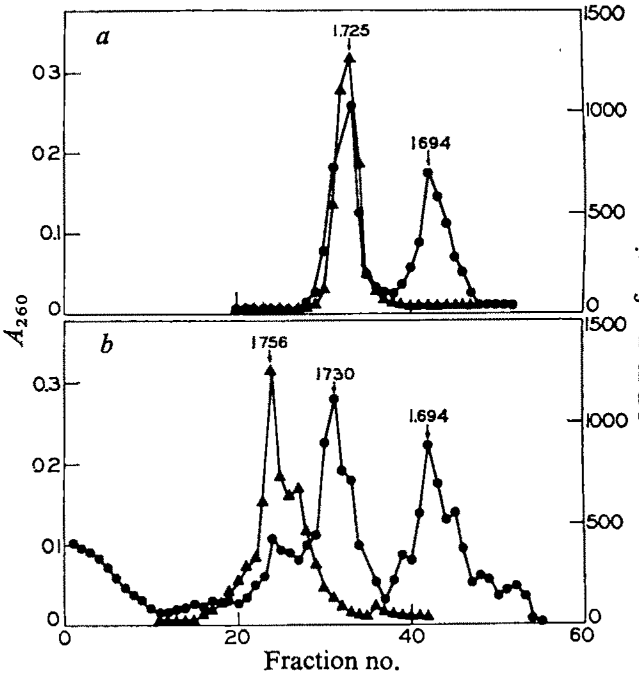


Fig. 2 Distribution in CsCl gradients of pulse labelled, sheared and denatured *E. coli* 15T$^-$ DNA (200 µg per sample) in the absence of any polyribonucleotide (*a*) and in the presence of 500 µg of poly(U) (*b*). DNA was extracted from synchronised, ^{3}H-thymidine-labelled cells, sheared, denatured and reacted with poly(U) as described in the text. The samples were centrifuged together with 120 µl of marker native *Cytophaga johnsonii* DNA (density in CsCl 1.694 g ml^{-1}, see ref. 1) in a Spinco SW 50.1 rotor, 38,000 r.p.m. at 8 °C. Fractions were collected at the end of the 48-h run. ●, Absorbance at 260 nm; ▲, radioactivity.

When this DNA is sheared into fragments shorter than the average distance between two adjacent clusters (breaks depicted by arrows in Fig. 1), denatured and reacted with a complementary ribopolymer, polyuridylic acid (poly(U)), fragments containing dA runs should band at the density higher than that of DNA alone (density of RNA is close to 1.9 g ml^{-1} in CsCl[14]). Fragments lacking dA runs should band at the normal density of denatured *E. coli* DNA (1.725 g ml^{-1} in CsCl[1,15]). (The unsheared DNA will band at the higher density since dA-T clusters are distributed evenly throughout *E. coli* DNA[1].) Thus, if a dA-T cluster (I in Fig. 1) is located between the two breaks, either inside or outside the labelled region, half of the labelled DNA (that strand which contains the dA run) should become heavier than the bulk of the unlabelled DNA in the presence of poly(U). If two dA-T clusters of opposite polarity (that is, with the dA segments present on opposite strands) are located close to 0, as are clusters I and II in Fig. 1, all the radioactive DNA will hybridise with poly(U) and become more dense than the bulk of the DNA, which is defined as the bulk of the ultraviolet-absorbing material. Thus, the experiment tells whether runs of dA are located close to the origin and whether they occur on one or both strands in that region.

Similarly, the occurrence of dC-dG clusters near the origin could be detected if polyriboguanylic acid (poly(G)) or the heteropolymer of guanylic and uridylic acids (poly(U,G)), is substituted for poly(U). Poly(U,G) has the same specificity as poly(G) but fewer technical problems are associated with its use[3,9,16].

A culture of *E. coli* 15T$^-$ (auxotrophic for thymine, arginine, methionine and tryptophan) was synchronised by the amino acid starvation technique[17]. The culture was supplemented with ^{3}H-thymidine (0.65 µCi, specific activity 1.56 Ci mmol^{-1}), immediately resupplied with the required amino acids and incubated for 15 s at 25 °C. Unlabelled thymidine was then added up to the concentration of 20 µg ml^{-1} and incubation continued for a further 2 min at 25 °C. The cells were chilled, collected and lysed by standard techniques. DNA was extracted[18] and divided into two portions, one of which was sheared by forcing the solution four times through a 27-gauge needle. Both DNA samples were then heat denatured[1] and each was divided further into three portions. Poly(U) was added to one portion and poly(U,G) to the second. The third contained no synthetic polynucleotide. A saturated solution of CsCl was added, the samples were centrifuged until equilibrium and the distributions of radioactive and ultraviolet-absorbing materials in the gradient were determined[3]. Some of the results are shown in Fig 2. Denatured DNA, not exposed to polyribonucleotide, banded in CsCl at a density close to 1.72 g ml^{-1} and the radioactive and ultraviolet-absorbing fractions practically overlapped (Fig. 2*a*). In the presence of poly(U) (Fig. 2*b*) the DNA came to equilibrium at a higher density and the ultraviolet-absorbing material was distributed over a wider range than in the control. All the radioactive DNA banded as a rather homogeneous peak well below the bulk of the DNA.

In several parallel experiments (not shown) the distribution patterns of the radioactive DNA and of the bulk of the unlabelled DNA in the gradient did not differ when: (1) the DNA was not sheared; (2) the pulse was longer than 15 s; (3) the pulse was begun more than 15 s after addition of the required amino acids; (4) either sheared or unsheared DNA was exposed to poly(U,G) instead of poly(U) and (5) DNA was obtained from non-synchronised cultures labelled as described.

The experiments summarised suggest that dA-T clusters (but not clusters of dC-dG) are located close to the replication origin in *E. coli* $15T^-$. Transposition by poly(U) of almost all the radioactive DNA from the density occupied by the bulk of cellular DNA suggests that at least two dA-T clusters of opposite polarity are located close to this point. The presence of a single heteropolymer composed of a run of dA residues followed by a run of Ts on the same strand, with complementary bases on the opposite strand, however, would account for the observed phenomena equally well.

We do not know how close these clusters are to the origin. They may be immediately adjacent to it, forming part of the sequence used by DNA polymerases to initiate DNA replication; or they may be as far as 500–700 nucleotide pairs away, half the length of the average DNA sheared in our conditions (unpublished observations). The latter estimate of this distance is probably too high in view of the relative homogeneity of the poly(U)-transposed radioactive peak (Fig. 2*b*). The fact that the dA-T clusters are also distributed throughout the DNA[1] may be related to repeated reinitiation of DNA synthesis[19].

The experiments described here do not suggest the precise role (if any) played by the dA-T clusters in DNA synthesis, although their location next to the starting point of replication may indicate some vital function. No information is available on the proximity of such clusters to the origin of DNA replication in other prokaryotic and eukaryotic cells. Identification of such structures in the vicinity of this site in DNA from other organisms would make a stronger case for their involvement in regulation of the replication process.

This work was supported in part by a grant from the US Public Health Service. B.B.B. was a recipient of a postdoctoral fellowship from the National Institutes of Health. We thank Guy Hoffman and Paul Sandok for technical assistance.

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Received February 24; revised April 4, 1975.

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Variable redundancy in RNA transcripts isolated in S and G_2 phase of the cell cycle of *Physarum*

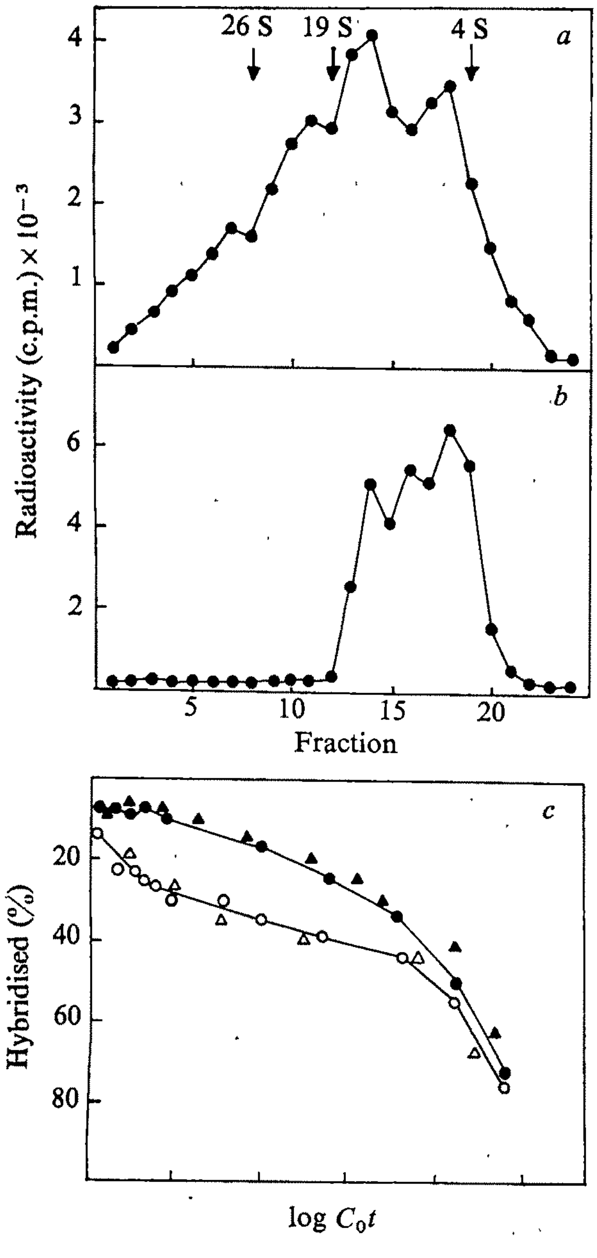
GENOMES of eukaryotes contain variable proportions of repeated base sequences of mostly unknown function[1]. It has been shown that nuclear RNA of sea urchin[2], rat[3] and *Dictyostelium*[4] also contains repetitive sequence elements, whereas polysomal messenger RNA (mRNA) does not. If mRNA is contained in the heterogeneous nuclear RNA, these sequence homology experiments suggest a selective processing of 3′-fractions of nuclear poly(A) RNA with unique base sequences. Most mRNA fractions and individual mRNAs so far studied[2,4-7] hybridise with single-copy DNA, with the exception of *Xenopus* embryo mRNA which contains both unique and moderately repeated sequences[8]. Functions for the repeated base sequences in RNA are not known and could involve regulation of transcription, processing, or translation, as well as copying of some sequences significant for DNA replication and recombination[1,9]. We have now used the naturally synchronous mitotic cycle of *Physarum*[10] to investigate whether there are changes in the degree of repeated sequences in RNA transcribed in S phase and G_2 phase.

We isolated poly(A)-rich RNA by affinity chromatography[11] from nuclei[12] and polysomes[13], and sedimented these molecules in sucrose gradients. The nuclear preparations were heterogeneous (Fig. 1*a*). They contained RNA fractions ranging from 4S to 30S, but no larger RNA molecules, whereas most of the poly(A)-rich RNA from polysomes sedimented with about 8S–15S (Fig. 1*b*). The RNA from nuclear preparations was then divided into RNA molecules of the size of polysomal RNA (Fig. 1*a*, fractions 13–22) and RNA molecules larger than polysomal RNA (Fig. 1*a*, fractions 1–12). Then the reassociation kinetics of these RNA molecules with DNA at an excess DNA concentration was determined[14]. We found that small molecules from nuclei and the RNA obtained from polysomes hybridised mainly with single-copy DNA (Fig. 1*c*) as judged from previous C_0t curves of nuclear DNA from *Physarum*[15]. A significant proportion of the heavy nuclear poly(A)-rich RNA had, however, hybridised at much lower C_0t values (Fig. 1*c*). To rule out hybridisation artefacts in comparing large and small RNA molecules, the heavy fraction of nuclear RNA was sonicated to about the size of polysomal RNA. The hybridisation kinetics of the shortened RNA molecules was indistinguishable from that of large RNA molecules (Fig. 1*c*).

From these observations we deduce that in *Physarum*, as in *Dictyostelium*[4], and perhaps in most higher cells[1], a fraction of nuclear RNA contains repeated base sequences which are lost in processing from the nucleus to the cytoplasm.

In the next experiment (and others not shown) we analysed the reassociation kinetics of nuclear poly(A)-rich RNA prepared in S phase or G_2 phase and found that the sample from G_2 phase contained a significantly higher proportion (35%) of redundant

Fig. 1 Sucrose gradient profiles and hybridisation kinetics of poly(A)-rich RNA from *Physarum polycephalum*. Plasmodia were grown[18] and labelled[11] as described previously, for 1 h with 100 μCi ml^{-1} ^{3}H-uridine. Nuclei[12] and polysomes[13] from *Physarum* were prepared using published methods. RNA was extracted with a phenol procedure adapted for *Physarum*[19–21], utilising trisisopropylnaphtalensulphonate for lysis and diethylpyrocarbonate as RNase inhibitor. Poly(A) RNA from nuclear and polysomal RNA was isolated by affinity chromatography on poly(dT) cellulose as reported previously[11,22] and fractionated on linear 5–20% sucrose gradients as published[11]. For hybridisation experiments aliquots of poly(A) RNA of about 10,000 c.p.m. were incubated with 1 mg ml^{-1} DNA in 2×SSC, 50 mM Tris, *p*H 7.2, 0.1% SDS in 50% formamide[3,14,23]. Nuclear DNA was prepared, purified, sheared and heat denatured as described previously[15]. At appropriate C_0t values the percentage of hybridisation was computed for each point from the radioactivity resistant to a mixture of RNase A and T1. At a C_0t value of 10^3 approximately 65–80% (700–900 c.p.m.) of the input RNA had hybridised. *a*, Profile of nuclear poly (A) RNA; *b*, profile of polysomal poly(A) RNA; *c*, hybridisation kinetics of poly(A) RNA from *Physarum*. ▲, Polysomal RNA; ○, large nuclear RNA molecules; △, large nuclear RNA, sonicated; ●, small nuclear RNA molecules.



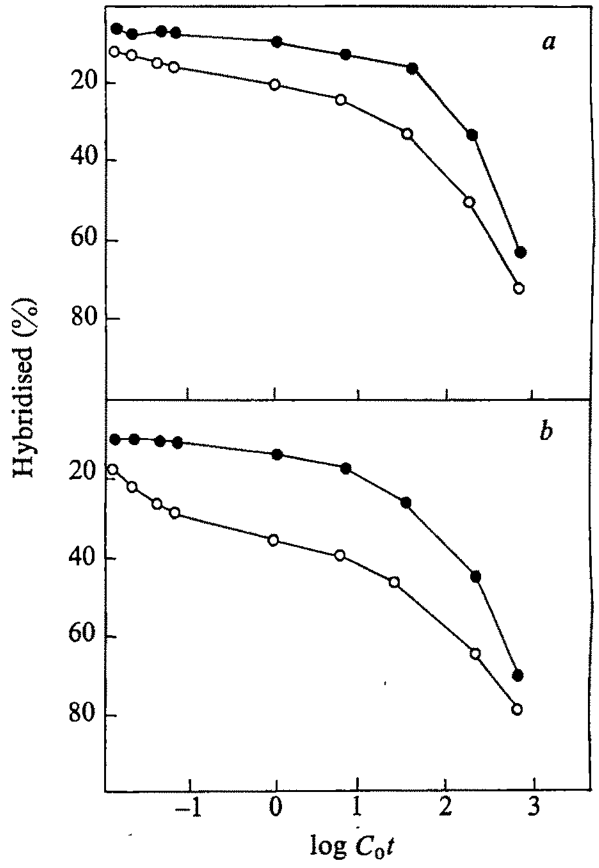


Fig. 2 Hybridisation kinetics of nuclear and polysomal poly(A) RNA prepared in S phase and G$_2$ phase. *Plasmodia* were labelled for 1 h in S phase (5–65 min post mitosis) and G$_2$ phase (4–5 h post mitosis). Preparation of subcellular poly(A) RNA fractions and hybridisation procedures were as described for Fig. 1. *a*, S phase of the cell cycle; ●, polysomal poly(A) RNA; ○, nuclear poly(A) RNA; *b*, G$_2$ phase of the cell cycle; ●, polysomal poly(A) RNA; ○, nuclear poly(A) RNA.

base sequences than that from S phase (20%) (Fig. 2). The hybridisation reaction of polysomal RNA prepared from S phase or G$_2$ phase was very similar (Fig. 2).

Differences in RNA synthesis in S phase and G$_2$ phase of *Physarum* have previously been claimed (for review see ref. 16) and we have since obtained some evidence for replication–transcription coupling[17]. The data presented in Fig. 2 suggest to us that replicating DNA may provide RNA polymerase B with other initiation sites for transcription than interphase chromatin, resulting in variable repetitive sequence elements in the nuclear RNA, possibly at the 5′-end of the original transcript. If one could generalise from this finding, we would predict that proliferating cells contain a lower degree of redundancy in their nuclear poly(A)-rich RNA than do resting cells.

This work was supported by the Deutsche Forschungsgemeinschaft.

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Received January 27; accepted April 3, 1975.

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Deletion of α-globin genes in haemoglobin-H disease demonstrates multiple α-globin structural loci

FOUR clinical syndromes are associated with α thalassaemia: (1) the silent carrier state (α thalassaemia-2), with no clinical effect; (2) heterozygous α thalassaemia (α thalassaemia-1) in which a more severe defect in α-chain synthesis produces microcytic red cells, but little or no anaemia; (3) haemoglobin-H disease, usually the combination of α thalassaemia-1 and α thalassaemia-2, resulting in mild to moderate haemolytic anaemia, and (4) hydrops fetalis associated with haemoglobin Barts, an invariably fatal condition which represents the homozygous state of α thalassaemia-1 (for recent reviews see refs 1 and 2). To explain these syndromes, one hypothesis proposes that α thalassaemia-1 and α thalassaemia-2 are caused by a defect of greater and lesser severity affecting alleles of a single α-globin locus[3]. A second hypothesis is based on evidence that the α-globin structural gene is duplicated[4], the four syndromes may be the result of involvement of one, two, three or all four α structural genes by thalassaemia. In this study, we tested these hypotheses by measuring the number of α-globin structural genes in haemoglobin-H disease and the results firmly support the latter hypothesis.

Ottolenghi *et al.* and Taylor *et al.* showed that the defect in hydrops fetalis results from complete or partial deletions affecting all α-globin stuctural genes[5,6]. This implies that in α thalassaemia-1, half the normal number of α-globin genes are similarly affected. Since some normal α-globin chain is synthesised in haemoglobin-H disease, an intact α-structural gene must be present in the α thalassaemia-2 lesion If there is one locus for α-chain synthesis, patients with haemoglobin-H disease will also have half the normal number of α-structural genes, but if haemoglobin-H disease is the result of deletion affecting three of the four genes, patients will have a quarter the normal number.

α-Globin genes were measured in a Chinese patient with haemoglobin-H disease with complementary DNA (cDNA) enriched in α-globin sequences. The specificities of this probe and the cDNA enriched in β-globin sequences are shown in Fig. 1. The α and β cDNAs annealed at the same rate and to the same extent to the haemoglobin-A mRNA, but the α cDNA annealed at about one-tenth the rate of the β probe to haemoglobin-H mRNA which contained $\alpha:\beta$ sequences at a ratio of 1 : 9. Less than a quarter of the α cDNA annealed to RNA from hydrops reticulocytes, whereas the β cDNA annealed completely. Thus, as much as 25% of the α cDNA used in these experiments could represent contamination with β-globin sequences.

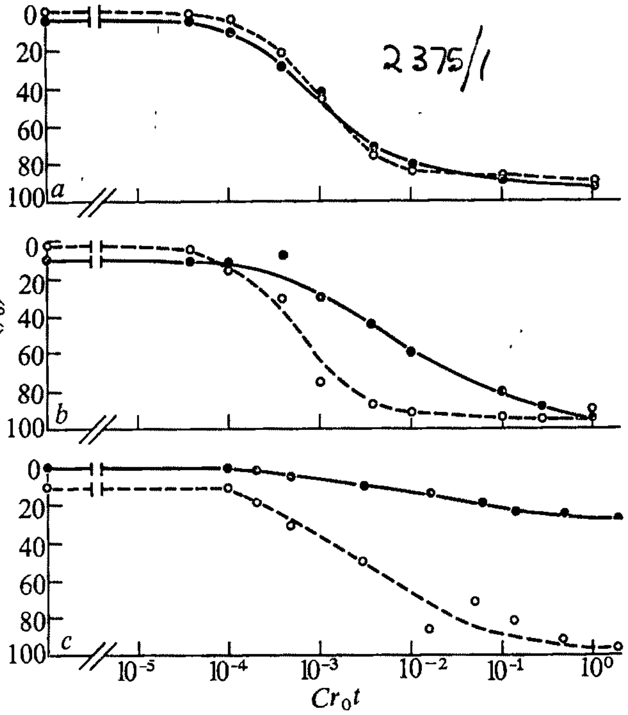

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Fig. 1 Annealing of human globin cDNA to globin mRNA. The α cDNA was prepared as described previously[6]. Globin mRNA was isolated from the postribosomal supernatant lysate[7] of reticulocyte-rich blood of a patient with autoimmune haemolytic anaemia. (This preparation of mRNA directed the synthesis of three times more α chain than β chain in the Krebs ascites cell-free system[3].) The mRNA was transcribed into cDNA in a 75-µl reaction mixture which contained 300 U ml^{-1} avian myeloblastosis virus polymerase, 20 µg ml^{-1} RNA template, 5 µg ml^{-1} oligo(dT) (PL Biochemicals), 100 µg ml^{-1} actinomycin (Calbiochem), 50 mM Tris (*p*H 8.3), 6 mM MgCl$_2$, 10 mM dithiothreitol (Calbiochem), 0.5 mM each of TTP, dCTP, dATP and 0.1 mM ^{32}P-dGTP (New England Nuclear Corp., specific activity 110 Ci mmol^{-1}); and incubated at 37 °C for 30 min. The cDNA was isolated and incubated in the presence of excess template RNA. That cDNA which annealed was isolated on hydroxylapatite and recovered as described[6]. It was then annealed to haemoglobin-H mRNA (α:β ratio = 1:9 on translation in Krebs ascites cell-free system), until 88% of the cDNA was annealed. The single stranded material was eluted from hydroxyl apatite and used as the probe for α sequences. The β cDNA was prepared by transcription from the haemoglobin-H mRNA, with ^{3}H-α GTP (Schwartz-Mann, specific activity 10 mCi mmol^{-1}) as the radioactive label, and was used as a probe for β sequences without further enrichment The purity of the α and β cDNA was tested by annealing to (*a*) Hb-A mRNA, (α: β = 1:1), (*b*) Hb-H mRNA and (*c*) to hydrops reticulocyte RNA. The conditions of annealing were similar to those described[6] except that 200 c.p.m. each of ^{32}P-α probe and ^{3}H-β probe were present together in each reacting mixture. ●, α cDNA; and ○, β cDNA. For (*c*) it was assumed that 1% of the total RNA was mRNA.

The contamination and the possible partial annealing of the α cDNA with the gene for ζ-globin chain[5] may complicate the measurement of α-globin genes. We attempted to circumvent these problems by measuring the rate of annealing of the cDNA to reconstituted mixtures of normal and hydrops cellular DNA. The rate of annealing of the α cDNA to a mixture containing equal parts of each should be similar to that found if half the normal number of α-globin genes are present. The rate of annealing to a mixture containing 1 part of normal to 3 parts of hydrops DNA would represent a quarter of the normal number of α-globin genes. These rates were compared with the rate of annealing of the α cDNA to cellular DNA from a patient with haemoglobin-H disease (Fig. 2). The α cDNA annealed to the haemoglobin-H DNA at a rate identical to the mixture which contained 25% non-thalassaemic cDNA and 75% hydrops DNA. In contrast, the cDNA specific for β-globin genes annealed to all the cellular DNAs and their mixtures at about the same rate.

We conclude that a patient with haemoglobin-H disease has a quarter the normal number of intact α-globin structural genes. This provides direct evidence that a non-thalassaemic person must have at least four α-globin genes per diploid cell. These data do not rule out the possibility that some multiple of four α-globin genes per cell is present. The fact that α-structural abnormalities constitute at least 25% of the total haemoglobin in the heterozygous state[9], however, makes the higher numbers unlikely.

On the basis of studies of α-globin structural mutants, it has been suggested that the number of α-globin structural genes varies in different populations. The finding of two α-chain mutants, haemoglobins Buda and Pest each comprising about 25%, together with 50% normal haemoglobin-A in the same person in a Hungarian family, firmly supports the two-locus theory[10]. On the other hand, the fact that haemoglobin-A is absent in Melanesian homozygous for haemoglobin J-Tongariki[11,12] and in some Oriental patients with the combination of haemoglobin Q and α thalassaemia-1 (ref. 13), suggests that only one locus is present. The method described here can be used to quantitate α-globin structural genes in these different syndromes.

We thank Professor J. M. Bishop for helpful comments.

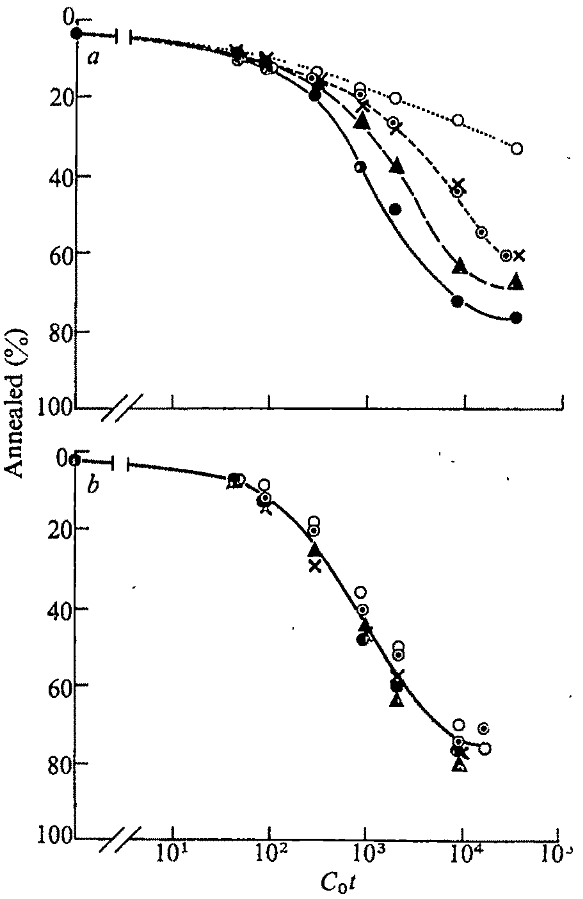

Fig. 2 Annealing of human globin cDNA to cellular DNA. DNA was isolated from the livers of an infant with hydrops and a non-thalassaemic infant, and from the spleen of a Chinese patient with haemoglobin-H disease by methods previously described[6]. The latter patient has a microcytic anaemia with haemoglobin of 10 g, reticulocyte count 4.6%, Hb A_2 0.9%, HbF 0.5%, Hb H 15% and no Hb Constant Spring. One sibling studied has heterozygous α-thalassaemia. 200 c.p.m. each of ^{32}P-α cDNA (*a*) and ^{3}H-β cDNA (*b*) were annealed to cellular DNAs in the following conditions: 5.8 mg ml^{-1} cell DNA, 0.5 M NaCl, 2 mM EDTA, 40 mM Tris (*p*H 7.4) at 68 °C, total volume 260 µl, for 10 min to 120 h. Annealing was assayed with hydroxylapatite. ●, Non-thalassaemic DNA; ○, hydrops DNA; ▲, mixture of equal parts of non-thalassaemic and hydrops DNA; ⊙, mixture of 1 part of non-thalassaemic to 3 parts of hydrops DNA; ×, haemoglobin-H DNA.

This work was supported by grants from the John A. Hartford Foundation, the National Institutes of Health, and Research career development awards (Y.W.K. and H.E.V.), from the US Public Health Service.

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Received January 13, revised March 24, 1975.

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Stereochemical basis of heat stability in bacterial ferredoxins and in haemoglobin A2

MOST enzymes are quickly inactivated above about 55 °C, but those from thermophile bacteria are stable for long periods at higher temperatures[1]. We do not know why, because so far their structures have proved too complex. For example, although the tertiary and quaternary structures of the enzyme glyceraldehyde phosphate dehydrogenase from lobster muscle and from *Bacterium stearothermophilus* are alike, their amino acid sequences differ by more than 130 out of some 330 positions, which makes it hard to decide why the stearothermophilus enzyme is more stable. The electron transfer protein ferredoxin offers a better chance, because its single polypeptide chain contains fewer than 60 residues; its structure is known, and its heat stability and amino acid sequence have been determined in both mesophile and thermophile bacteria. We have built an atomic model of this protein, replaced its amino acid side chains in turn to correspond to the published sequences and searched for possible causes of the greater heat stability of ferredoxins from thermophile bacteria. We found that it arises mainly from external salt bridges linking residues near the amino terminus to others near the carboxy terminus. Haemoglobin A_2, a minor fraction of adult human haemoglobin which is a little more heat stable than the major fraction, haemoglobin A, seemed another good choice because its amino acid sequence differs from that of A at only 10 positions. The atomic model suggests that at only two of these positions are the replacements likely to contribute to the extra stability of haemoglobin A_2, one replacement providing an extra hydrogen bond between the α_1 and β_1 subunits and the other adding two non-polar interactions to a surface crevice within the β subunits To account for the increased heat stability of the two proteins, the extra bond energy

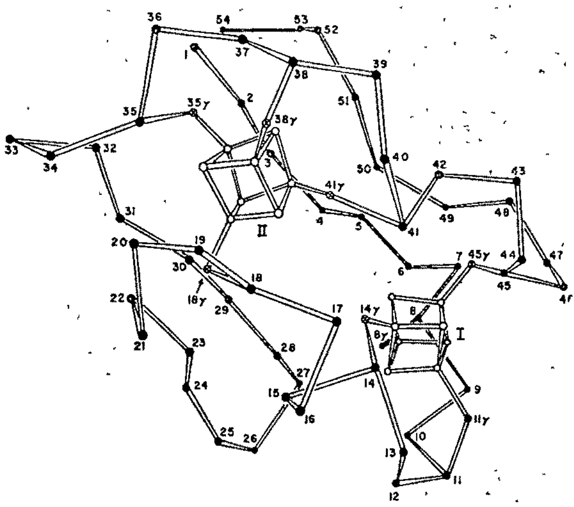

Fig. 1 Plot of α-carbon, iron and sulphur positions in ferredoxin from *M. aerogenes*[2]. (Reproduced by permission of *J. biol. Chem.*) Fe⊙; S_{inorg}○; S_{Cys}×; C_α●.

provided by these interactions need not be larger than 10 kJ for ferredoxin or 5 kJ for haemoglobin A_2.

The three-dimensional structure of ferredoxin from the mesophile *Micrococcus aerogenes* has been determined[2] (Fig. 1). It has two active complexes spaced 12 Å apart, each consisting of four atoms of iron and four of inorganic sulphur joined in a distorted cube. The iron atoms of one complex are linked to Cys 8, 11, 14 and 45 and those of the other to Cys 18, 35, 38 and 41. The molecule contains tyrosines in positions 2 and 28, each having one face in contact with one of the Fe_4S_4 complexes and one edge with water.

The thermal stabilities of ferredoxins from two mesophile clostridia (*Clostridium pasteurianum* and *C. acid urici*) and from two thermophile species (*C. tartarivorum* and *C. thermosaccharolyticum*) have been reported[3]. The amino acid sequences of these four ferredoxins are closely related to the sequence of *M.aerogenes* (Table 1). For instance, the sequences of *M. aerogenes* and *C. thermosaccharolyticum* show thirty identities, including the pattern of the eight cysteines which are linked to the eight iron atoms. There are ten conservative replacements (for example Ala→Ser; Ser→Thr; Thr→Val; Val→Ile; Gln→Glu) and in eight of these the side chains are external; the two internal replacements are Tyr 2→His at one of the active sites, and Ile→32 Val; both these can be accommodated without change of tertiary structure. There are 12 non-conservative replacements in 11 of which the side chains are external; the one internal replacement Pro 52→Val can be accommodated without change of tertiary structure. Compared to the sequence of *M. aerogenes* the sequences of the four *Clostridium* ferredoxins contain two insertions, one between positions 21 and 22 and the other between 26 and 27, and one deletion at the C-terminus. Fig. 1 shows that the insertions occur at the corners of an external loop and that the C-terminus is also external, so that these modifications can also be accommodated without changing the tertiary structure which seems to be determined primarily by the pattern of cysteines linked to the Fe_4S_4 complexes.

The enzyme from *C. pasteurianum* is completely inactivated after 2 h at 70 °C, that from *C. acidi urici* retains 20% of its activity, that from *C.tartarivorum* half, and that from *C thermosaccharolyticum* 90% (ref 3). To explain these differences we have assumed that the polypeptide chains of all four clostridia maintain a tertiary structure closely similar to that of *M. aerogenes*, we have built an atomic model of the latter and have altered the pattern of side chains in turn to correspond to the four different sequences of *Clostridium*. We have found some of the amino acid replacements such as Thr 5→Asn or Thr 49→Val to be clearly neutral, although others seem highly significant.

For instance, the nature of the aromatic residues in positions 2 and 30, next to the two Fe_4S_4 complexes, varies in different species (Table 2). Ferredoxin from *C. pasteurianum*, which is the least heat stable, has Tyr at 2 and Phe at 30. In *C. acidi urici*, which is more stable, Phe 30 is replaced by Tyr; its OH can form a hydrogen bond with the OH of Ser 10. In *C. tartarivorum* and *C. thermosaccharolyticum* Tyr continues to occupy position 30, but Tyr 2, which seems to make no hydrogen bond, is replaced by His which can make a hydrogen bond to the sulphur lone pair electrons of Cys 43. Tanaka *et al.* drew attention to some of the most interesting characterisics of the thermophile enzymes

Table 1 Amino acid sequences of four mesophile and two thermophile ferredoxins

	1	$\boxed{2}$	3	$\boxed{4}$	5	$\boxed{6}$	7	$\boxed{8}$	$\boxed{9}$	10	$\boxed{11}$	$\boxed{12}$	$\boxed{13}$	$\boxed{14}$	15	16	17	$\boxed{18}$	$\boxed{19}$	$\boxed{20}$	21	22	$\boxed{23}$	24	$\boxed{25}$	$\boxed{26}$	27	28	29
M. aerogenes[9]	A	Y	V	I	N	D	S	C	I	A	C	G	A	C	K	P	E	C	P	V	N	–	I	Q	Q	G	–	S	I
C. pasteurianum[7]	A	Y	K	I	A	D	S	C	V	S	C	G	A	C	A	S	E	C	P	V	N	A	I	S	Q	G	D	S	I
C. acidi urici[8]	A	Y	V	I	N	E	A	C	I	S	C	G	A	C	D	P	E	C	P	V	D	A	I	S	Q	G	D	S	R
P. elsdenii[13]	M	H	V	I	S	D	E	C	V	K	C	G	A	C	A	S	T	C	P	T	G	A	I	E	E	G	E	T	K
C. tartarivorum[10]	A	H	I	I	T	D	E	C	I	S	C	G	A	C	A	A	E	C	P	V	E	A	I	H	E	G	T	G	K
C. thermosaccharolyticum[11]	A	H	I	I	T	D	E	C	I	S	C	G	A	C	A	A	E	C	P	V	E	A	I	H	E	G	T	G	K

	$\boxed{30}$	31	$\boxed{32}$	33	34	$\boxed{35}$	$\boxed{36}$	$\boxed{37}$	$\boxed{38}$	$\boxed{39}$	$\boxed{40}$	$\boxed{41}$	42	43	44	45	$\boxed{46}$	$\boxed{47}$	$\boxed{48}$	$\boxed{49}$	50	$\boxed{51}$	52	53	54	55	56
M. aerogenes[9]	Y	A	I	D	A	D	S	C	I	D	C	G	S	C	A	S	V	C	P	V	G	A	P	N	P	E	D
C. pasteurianum[7]	F	V	I	D	A	D	T	C	I	D	C	G	N	C	A	N	V	C	P	V	G	A	P	V	Q	E	–
C. acidi urici[8]	Y	V	I	D	A	D	T	C	I	D	C	G	A	C	A	G	V	C	P	V	D	A	P	V	Q	A	–
P. elsdenii[13]	Y	V	V	T	–	D	S	C	I	D	C	G	A	C	E	A	V	C	P	T	G	A	I	S	A	E	–
C. tartarivorum[10]	Y	Q	V	D	A	D	T	C	I	D	C	G	A	C	Q	A	V	C	P	T	G	A	V	K	A	E	–
C. thermosaccharolyticum[11]	Y	E	V	D	A	D	T	C	I	D	C	G	A	C	E	A	V	C	P	T	G	A	V	K	A	E	–

Homologies and conservative replacements are boxed. The one-letter amino acid code is as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln, R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr.

Table 2 Aromatic residues in positions 2 and 30 of ferredoxins

	Thermolabile				Thermostable	
Residue no.	*M. aerogenes*	*C. pasteurianum*	*C. acidi urici*	*P. elsdenii*	*C. tartarivorum*	*C. thermosaccharolyticum*
Hydrogen bonds gained						
2	Tyr	Tyr	Tyr	His	His	His
43	Cys	Cys	Cys	Cys	Cys	Cys
10	Ala	Ser	Ser	Lys	Ser	Ser
30	Tyr	Phe	Tyr	Tyr	Tyr	Tyr
Invariant salt bridge						
1	Ala	Ala	Ala	Met	Ala	Ala
39	Asp	Asp	Asp	Asp	Asp	Asp
Salt bridges gained						
2	Tyr	Tyr	Tyr	His	His	His
6	Asp	Asp	Glu	Asp	Asp	Asp
7	Ser	Ser	Ala	Glu	Glu	Glu
24	Gln	Ser	Ser	Glu	His	His
25	Gln	Gln	Gln	Glu	Glu	Glu
29	Ile	Ile	Arg	Lys	Lys	Lys
31	Ala	Val	Val	Val	Gln	Glu
44	Ala	Ala	Ala	Gln	Glu	Glu
53	Asn	Val	Val	Ser	Lys	Lys

The salt bridge from Asp 6 to Lys 29 may not be sterically favourable and is therefore shown as a broken line. The numbering of the residues is as in Table 1; to relate it to Fig. 1, subtract 1 from numbers 23–26 and 2 from all numbers above 27. Ferredoxin from *P. elsdenii* is reported to be heat labile[13] but no quantitative data for its heat stability are available. On the basis of its salt bridges as shown here it should be intermediate between *C. acidi urici* and *C. tartarivorum*.

when they pointed out that glutamine or glutamic acid in positions 31 and 44 can be hydrogen bonded to other amino acid side chains. In fact, glutamates 7 and 25, glutamate or aspartate 6, and aspartate 39 can also form such bonds. Aspartate 39 forms a salt bridge with the α amino group which seems to be invariant. Two of the salt bridges specific to the thermophile enzymes (24–25 and 29–31) are between neighbours or next nearest neighbours in straight segments of the chain and would not necessarily have to break when the chain unfolds, but two others are placed in the most effective positions possible by clamping residues near the amino terminus to residues near the carboxyl terminus (His 2–Gln or Glu 44 and Glu 7–Lys 53). The sequence of the less stable ferredoxin from *C. tartarivorum* differs from that of the more stable one from *C. thermosaccharolyticum* by replacements in only two positions. Glutamines 31 and 44 are replaced by glutamates. His 2 and Lys 29, which are common to the ferredoxins of both clostridia, can form hydrogen bonds with either the uncharged carbonyls of the glutamines or the charged carboxyls of the glutamates; the latter, being salt bridges, are stronger and must therefore account for the extra stability of the ferredoxin from *C. thermosaccharolyticum*. If these conclusions are correct, then the differences between the heat stabilities of the different ferredoxins should diminish on weakening the salt bridges, for example with increasing *p*H which would discharge histidine 2, or with increasing ionic strength.

In addition to these polar interactions one might have expected the thermophile enzymes to show extra non-polar interactions, but comparison of the sequences does not point to any position where the thermophile enzymes contain a residue providing consistently more non-polar contacts than any of the amino acid side chains occupying the same position in the mesophile enzymes.

We now turn to human haemoglobin, a protein consisting of four subunits joined in a tetrahedral array. At 45° oxyhaemoglobin A_2 is denatured six times more slowly than A (ref 4). The two proteins differ in only ten positions in the β chains (known as δ in haemoglobin A_2) (Table 3). Seven of these are external, two lie in surface crevices and one lies in the $\alpha_1\beta_1$ contact. Analysis of the external replacements shows that their effect, if any, should be to stabilise haemoglobin A rather than A_2. One replacement (126) in a surface crevice provides an additional non-polar contact between helices A and H within the same β chain of haemoglobin A_2. The replacement in the $\alpha_1\beta_1$ contact (116), introduces one extra hydrogen bond between the guanidinium group of Arg G18(116)β_1 and the carbonyl oxygen of Pro GH2(114)α_1 of haemoglobin A_2 (for illustration of the $\alpha_1\beta_1$ contact see refs 5 and 6.) Dissociation at the $\alpha_1\beta_1$ contact seems to determine the degree of resistance to alkaline denaturation of haemoglobin[6]. The amino acid differences between haemoglobins A and A_2 suggest that the extra hydrogen bond between residues 114α_1 and 116δ_2 is decisive in making A_2 more resistant to heat denaturation. If this is correct, then haemoglobin Lepore Boston, a δ–β hybrid in which the N-terminal six residues are δ and the rest β, should have a susceptibility to heat denaturation

Table 3 Interactions in human haemoglobin A and A_2

Residue no.		A β	A_2δ	Position	Structural role
Probable gains in A_2					
116	G18	His	Arg	$\alpha_1\beta_1$ contact	Arg forms H-bond to CO of Pro GH2α
126	H4	Val	Met	Surface crevice	Additional non-polar contact with Val A11(14)β
Probable losses in A_2					
12	A9	Thr	Asn	Surface crevice	Thr OH may bond to CO of Lys A5β
22	B4	Glu	Ala	External	Glu may form weak salt bridge to His G19β
50	D1	Thr	Ser	External	Possible loss of non-polar contact with CH_3 of Ala D4
117	G19	His	Asn	External	See 22
Probable neutral replacements					
9	A6	Ser	Thr	External	None
86	F2	Ala	Ser	External	Probably none
87	F3	Thr	Gln	External	None
124 or 125	H2 or H3	Pro	Gln	External	None

similar to that of haemoglobin A, and deoxyhaemoglobin, in which the subunits are joined by salt bridges, should be more heat-resistant than oxy, CO or cyanomethaemoglobin.

In conclusion, thermophile ferredoxins are stabilised by extra salt bridges between external polar groups and by additional hydrogen bonds but not, apparently, by more non-polar contacts. The heat stability of haemoglobin A_2 compared to A is accounted for by one extra hydrogen bond at the contact between the α_1 and δ_1 subunits and possibly two non-polar interactions within the δ subunits.

The denaturation data of ferredoxin refer to irreversible loss of activity accompanied by loss of the iron sulphur clusters from the protein; this requires at least two kinetic steps, unfolding of the chain and dissociation of the cluster, which cannot be separated. Even so the order of magnitude of the extra stabilisation energy provided by the salt bridges and hydrogen bonds can be estimated from the difference in the observed rates of denaturation. The data of Devanathan *et al.*[3] suggest that the half times for denaturation ($t_{1/2}$) in the four proteins differ approximately in the order of 1 : 5 : 10 : 20. The corresponding differences in free energy of activation, $\Delta G = RT2.3\ \log\Delta t_{1/2}$ amount to 4.6, 6.6 and 8.5 kJ mol^{-1} at 70 °C for each protein relative to the slowest, assuming G (ground state) to be the same for each protein. This suggests that the total extra free energy of stabilisation provided by the additional bonds in the thermophile ferredoxins is rather less than 10 kJ mol^{-1}. The rate of denaturation at 45 °C of haemoglobin A exceeds that of A_2 about sixfold, which corresponds to an extra stabilisation energy of 5 kJ mol^{-1}. These energies are surprisingly small. Similar conclusions were reached for the difference between the free energy of activation needed to denature triose phosphate isomerase from rabbit muscle and *B.stearothermophilus* (R. C. Fahey and J. I. Harris, unpublished). It seems that in monomeric enzymes the extra energy of stabilisation can be provided without disturbance of the tertiary structure by a few extra salt bridges on the molecular surface; in oligomeric enzymes by some extra salt bridges, hydrogen bonds or non-polar bonds at the subunit interfaces.

We thank Drs Lyle Jensen, L. C. Sieker and E. T. Adman for allowing us to use their unpublished coordinates of ferredoxin and for checking our conclusions; and Drs J. Kinderlehrer, M. Levitt and A. Warshel for discussion.

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Received March 1; accepted April 7, 1975.

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Isolation of newly synthesised myosin filaments from skeletal muscle homogenates and myofibrils

LARGE and rapid changes in the concentrations of myofibrillar protein can occur during hypertrophy and atrophy of skeletal and cardiac muscle[1,2]. Understanding of the regulation of these changes will require analysis of the steps between the initiation of transcription and the degradation of the protein to amino acids. The role of post-translational steps in these processes has generally been ignored since there has been no way to isolate biochemically intermediates of myofibrillar assembly. This is unfortunate since the physiological and metabolic regulation of such steps may control the rate of incorporation of new protein into this organelle, and thus the levels of functional contractile protein in the cell. Present understanding of myofibrillar assembly has come mainly from ultrastructural studies. In embryonic[3], regenerating[4] and rapidly growing muscles from young animals[5], electron microscopy suggests that polymerised thick and thin filaments can aggregate to form myofibrils *de novo* and/or insert into pre-existing myofibrils. In contrast, microscopy of normal adult muscle usually does not reveal these structural aspects of myofibrillogenesis, although isotope studies showed that contractile proteins are constantly synthesised and degraded in the muscle cell[1,2,6], even in the absence of net growth. This may be because only a small fraction of protein exists as nascent filaments not yet integrated into fibrillar sarcomeres, and/or because these assembly steps may be too rapid to be captured by electron microscopy. We report here the isolation of a population of thick and thin myofilaments that may constitute such an intermediate in myofibrillar assembly.

Female Sprague–Dawley rats (200–220 g) were killed and the muscles excised, minced and soaked in an EGTA-pyrophosphate containing relaxing solution, and then homogenised in a solution containing EGTA. The homogenate was centrifuged at 800*g* to sediment myofibrils and centrifugation was repeated until the supernatant was devoid of myofibrils. Electron microscopy of this fraction revealed the presence of numerous free thick and thin filaments which tended to form 'bundles' through lateral and longitudinal aggregation. By supplementing the post-myofibrillar supernatant with 2 mM ATP, the aggregation of these myofilaments could be prevented, in turn making clearer visualisation of these filaments possible by negative staining and the subsequent centrifugation of this fraction at 10,000*g* for 10 min to remove additional subcellular material from this fraction. Finally, these filaments were purified further by treatment with 1% Triton X-100 based on previous reports that no myofibrillar components are extracted with this non-ionic detergent[7]. Figure 1 illustrates a field of such filaments after this purification. The amount of protein in this preparation, measured by the Lowry method[8], varied with the degree of homogenisation of the muscle but was between 10% and 15% of total myofibrillar protein when homogenisation was carried out for 30 s at 14,000 r.p.m. in an MSE blade type homogeniser.

Since the release of filaments in the whole muscle homogenate is likely to depend in part on endogenous muscle ATP necessary for actin-myosin relaxation[9], we quantified the amount of myofibrillar protein found in the purified filaments as a function of the time that minced muscle was soaked before homogenisation. When this step lasted 3 h, with repeated replacement of the washing solution, virtually no protein was detected in this preparation and no free filaments or aggregated filaments were found in the crude muscle homogenate examined with the electron microscope. Thus, the free and/or loosely held filaments are probably trapped on the surface of the myofibrils through, in part, rigour-links caused by washing out of endogenous ATP from the mince. This suggested an alternative method for isolating fresh homogenate filaments, based on their entrapment on myofibrils and subsequent release. Myofibrils were, therefore, prepared from this 'rigour homogenate' and gently treated with Mg-ATP relaxing solution, resulting in the release of approximately 10–15% of myofibrillar protein in the form of thick and thin filaments. When myofibrils were viewed with the phase contrast or electron microscope during treatment with Mg-ATP relaxing solution, they seemed at first to stiffen, followed by a swelling and fanning out of filaments at the periphery of the fibrils, suggesting the release of filaments from the fibrillar periphery. In related studies, we have shown that the whole homogenate filaments and filaments released from fibrils lack M-line protein(s) of 190,000 and 170,000 daltons and Z-line protein(s) of 90,000 daltons, and have decreased amounts of protein larger than 200,000 daltons (ref. 7 and J. D. E., unpublished).

To investigate the significance of the easily releasable filaments, the rats were first injected with tritiated leucine into the tail vein after a 24 h fast. After times ranging from 5 min to 24 h, the animals were killed and the isolated filaments were prepared from the whole homogenate directly or through entrapment on the myofibrils and subsequent filament release. The residual undispersed myofibrils were also prepared in each case. The preparations were electrophoresed on 5% SDS-acrylamide gels and the stained myosin heavy chain band was sliced and hydrolysed (Fig. 2). Tritium was counted in one portion of the hydrolysate and the remainder was assayed for leucine (Table 1).

The specific activity of the myosin heavy chain in the filament fractions was substantially and reproducibly higher than that obtained from residual myofibrils (Table 1). Furthermore, the differences in these specific activities seemed to decrease with time after isotope injection. The precise time dependence of this difference must, however, be regarded as tentative because small variations in filament yield may affect the exact difference obtained in a given experiment. We have also obtained similar results in the heart (J. Sims and R. Z., unpublished). The quantitation of this phenomenon and its applicability to other myosin and myofibrillar components is being studied.

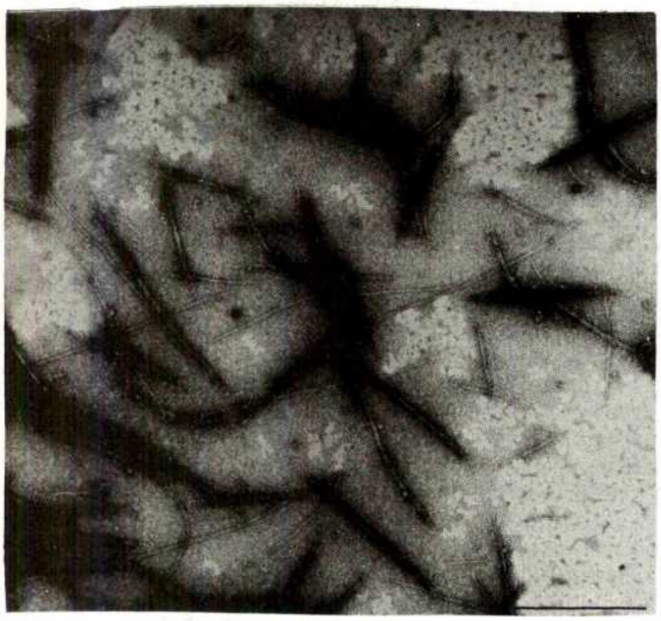

Fig. 1 Electron micrograph of thick and thin filaments isolated from the post-myofibrillar supernatant fraction of a fresh muscle homogenate. Mixed rat back muscle was excised and coarsely minced in 0.1 M KCl, 2 mM $MgCl_2$, 2 mM EGTA, 2 mM $Na_4P_2O_7$, 1 mM dithiothreitol (DTT), 0.01 M Tris-maleate, *p*H 6.8 at 0 °C. The muscle was then disrupted with an MSE homogeniser for 30 s at 14,000 r.p.m. in 10 volumes of a solution of the same composition without pyrophosphate, followed by 5–10 strokes in a glass Dounce homogeniser. The homogenate was filtered through two layers of cheesecloth and centrifuged at 800*g* for 10 min. This supernatant was centrifuged again at 800*g* for 10 min and then adjusted to a final ATP concentration of 2 mM to prevent aggregation of myofilaments. The ATP-supplemented filament preparation was then centrifuged at 10,000*g* for 10 min. The post-10,000*g* supernatant was then supplemented with Triton X-100 to yield a final concentration of 1%, and placed on ice for 5 min. Next $CaCl_2$ was added to a final concentration of 2 mM and, after 15 min, the aggregated filaments were pelleted by centrifugation at 15,000*g* for 1 h. These filaments were then redispersed in 0.1 M KCl, 2 mM $MgCl_2$, 2 mM EGTA, 2 mM ATP, 1 mM DTT, 0.01 Tris-maleate, *p*H 6.8 (Mg-ATP relaxing solution) and negatively stained with aqueous 2% uranyl acetate. The calibration bar equals 0.5 μm.

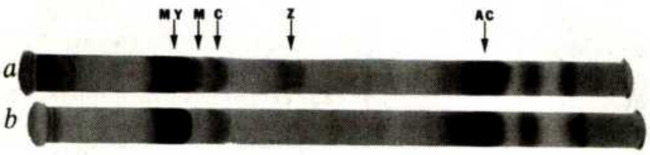

Fig. 2 SDS gel electrophoresis of filaments and residual myofibrils isolated from a fresh muscle homogenate. Isolated filaments were prepared as described in Fig. 1 and the 800*g* pellet obtained from the muscle homogenate was used to obtain myofibrils. The myofibrils were prepared by washing the pellet six times with 0.1 M KCl, 2 mM $MgCl_2$, 2 mM EGTA, 1 mM DTT, 0.01 M Tris-maleate, *p*H 6.8, once with the same solution containing 1% Triton X-100, and twice without Triton. Each preparation was then electrophoresed on 5% SDS-acrylamide gels as described previously[12]. Similar patterns are obtained when filaments are released from fibrils although the proportions of myosin and actin varied between preparations. A, residual myofibrils; B, thick and thin filaments; MY, myosin heavy chain; M, M-line protein(s); Z, Z-line protein(s); C, C-protein; AC, actin. Assignment of protein bands is according to ref. 7.

In most protocols for the isolation of contractile proteins, the 'insoluble' myofibrillar fraction serves as the starting point for extraction and purification. Discarding the supernatant fraction which we have found to contain myosin filaments of substantially higher specific activity could seriously affect estimates of protein synthetic rates and protein half lives based on these rates. In the whole animal system used here, apparent rates of synthesis would seem lower than the actual rates, particularly at the earlier time points[10].

The myofilaments found in the supernatant fraction must be either filaments which are not as tightly incorporated into

Table 1 Standard specific radioactivity of the myosin heavy chain in filament fractions and in residual myofibrils

Experiment	Time after injection	Filaments (standard specific activity)	Residual myofibrils (standard specific activity)
1	5 min	2,648	767
2	5 min	3,620	695
3	5 min	1,453	586
4	30 min	2,263	852
5	3 h	2,873	1,513
6	24 h	2,885	2,027

Rats were injected with 300–2,000 μC; of ^{3}H-leucine. In experiments 1 and 2, filaments and myofibrils were isolated directly from the fresh whole muscle homogenate as described in Fig. 1, Fig. 2 and in the text. In experiments 3 to 6, filaments were released from 'rigour homogenate' myofibrils. Filaments were released from the myofibrils by 10 passages through a Pasteur pipette of the fibrils in Mg-ATP relaxing solution (Fig. 1). The filaments were separated from undispersed fibrils by centrifugation at 800*g* for 10 min after which the supernatant was recentrifuged at 800*g* for 10 min. The filaments in the 800*g* supernatant were pelleted by centrifugation at 100,000*g* for 3 h. The myosin heavy chain band (MY) was sliced from the stained gels (Fig. 2). A 3 mm slice containing 20–50 μg protein was hydrolysed in 5 ml of 6 N HCl–0.1% mercaptoethanol at 110 °C for 24 h. The samples were then placed at 0 °C for about 12 h and then centrifuged at 500*g*. The supernatant was evaporated to dryness and the precipitate was washed three times with H_2O by evaporation. Samples were resuspended with 1.3 ml of H_2O and then 1 ml was counted in 10 ml of Scintisol with a Packard liquid scintillation counter. 0.2 ml was used to assay for leucine according to the isotope dilution method of Rubin and Goldstein[13]. Results are expressed as standard specific activity, = d.p.m. μmol^{-1} leucine × rat weight (g) per μCi injected.

the fibrils or filaments which actually exist in a cytoplasmic pool before their attachment to fibrils. (Since the amount of myofibrillar protein recovered in the supernatant fraction at least partly depends on the extent of tissue disruption, a portion of these filaments must be released from myofibrils.) Further experiments do not support the hypothesis that the high specific activity filaments arise from the preferential release of filaments from the myofibrils of red compared with white fibres (our work in preparation).

Morkin's electron microscopic autoradiographic studies of postnatal skeletal muscle suggested that newly synthesised myofilaments are added to the periphery of pre-existing fibrils[11]. It is likely that we have, at least in part, obtained a preferential separation of these newly synthesised peripheral filaments from the less dissociable 'core' myofibrils. In non-growing heart muscle or skeletal muscle undergoing little or no increase in length, peripheral addition would seem to be a reasonable way to add new myofilaments. In hypertrophying heart muscle or in skeletal muscles from prenatal or young animals, which are rapidly lengthening along the fibre axis, addition of new filaments to the ends of myofibrils[5,14] may also be important.

This work was supported in part by the US Public Health Service, the University of Chicago Cancer Research Center and the Muscular Dystrophy Associations of America, and by the Chicago and Illinois Heart Associations. We thank Mrs Gwen Szent Gyorgi and Dr Ann Martin for assistance. The Franklin McLean Memorial Research Institute is operated by the University of Chicago for the US Energy Research and Development Administration.

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A similar law may govern water freezing in minerals and living organisms

THERE is an interesting similarity between the freezing of water in minerals and in biological tissues. Mazur[1,2] observed that many living organisms lose viability at threshold temperatures near −10 °C. After investigating this phenomenon from the point of view of possible mechanisms by which living cells suffer damage on freezing, he concluded that a key step in the freezing process is the penetration of ice into the cell to nucleate the internal water, and that at −10 °C ice can penetrate the membrane, whereas above this temperature, it cannot. This implies that the water in the cell-membrane pores or a portion of them, starts to freeze at about −10 °C. From the Kelvin equation which describes freezing in terms of the ice–water surface tension and the radius of cylindrical capillaries, Mazur deduced that[1] "the lower melting point of ice in a capillary can account for the barrier properties of the plasma membrane". He observed, however, that "there is a large quantitative discrepancy. The evidence suggests that the plasma membrane ceases to be a barrier at approximately −10 °C. On the basis of equation (4), (ref. 1) the critical pore radius at −10 °C is 30 Å, whereas the estimated pore size from permeability studies is 3–8 Å". To account for the discrepancy he postulated that the contact angle of water in the membrane pores is not 0 ° but 80 °. This enabled him to find the simple relationship

$$\Delta T \approx 52/a \tag{1}$$

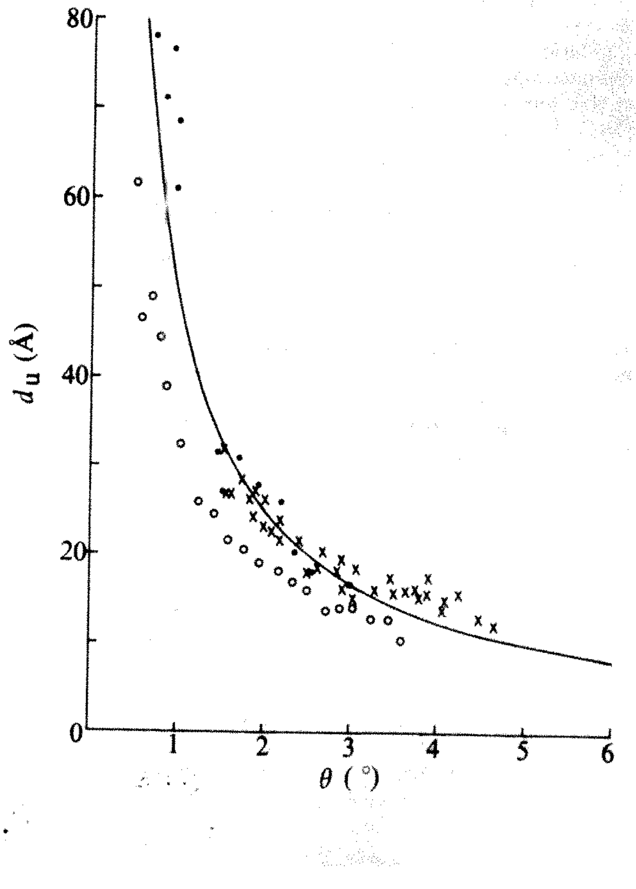
where ΔT is the freezing point depression and a is the pore radius in Å, which predicts that water in 5 Å pores will freeze at −10 °C and thus agrees with experimental observations on membrane pore sizes.

This result is strikingly similar to an equation that we have found governs the thickness of the unfrozen water layer, d_u, on mineral surfaces:

$$d_u \approx 50\ (1/\theta) \text{ or, rearranged, } \theta \approx 50/d_u \tag{2}$$

where d_u is in Å and θ is temperature in degrees below 0 °C; d_u is calculated from experimental results and is equal to the ratio of the volume of unfrozen water V, to the specific surface area of the solids S. For non-porous adsorbents or for porous systems in the unsaturated state, that is, when their pores are not completely filled with the adsorptive (a substance capable of being adsorbed but which is not in the adsorbed state) or adsorbate, d_u is considered to be the statistical average thickness of the layer of unfrozen water. In saturated porous systems the ratio V/S is equal to the hydraulic radius, r_h of the pores. For cylindrical capillaries, often used as convenient but probably not

Fig. 1 Measured relationships between the average statistical thickness of the layer of unfrozen water (d_u) and temperature below 0 °C (θ) for three different mineral materials. Also included is the smoothed theoretical curve fitted to the results (continuous line, $d_u = 50\,\theta^{-1}$). ×, Kaolinite; ●, basalt; ○, limonite–goethite.



very realistic models of pores, $r_h = r/2$ where r is the capillary radius. For slit-type capillaries, $r_h = d$ where d is half the distance of separation between the plates; slit-type capillaries are a reasonable model for pores between plate-shaped minerals such as the alumino-silicates. The value of the coefficient in equation (2) was derived from measurements[3] of the unfrozen water content as a function of temperature on several partially frozen, water-saturated, mineral–water mixtures, each differing quite considerably in their crystallographic structure and physicochemical properties. These data for powdered basalt (a primary, basic rock), limonite–goethite (iron oxide) and kaolinite (an aluminosilicate mineral) are depicted in Fig. 1. The fitted curve is simply a plot of equation (2). We discuss elsewhere how deviations from this freezing law may arise in porous bodies as a result of differences in distribution of pore size, and how these may affect the calculated thickness of unfrozen water layers at temperatures near 0 °C. At lower temperatures, however, (−6 to −10 °C) when the thickness of the unfrozen water layer lies in the range of 5–8 Å, water–ice phase composition data for most mineral materials fall on the same temperature–thickness curve. Note that the similarity between these three different materials would not have been as apparent if instead of layer thickness, unfrozen water content was plotted as a function of θ using the more common units of g H_2O/g solid, because of differences in specific surface areas between the various materials.

We will further analyse the possible physicochemical basis for the freezing process. Our hypothesis may be elaborated as follows: In the force field caused by the forces emanating from the solid or resulting from surface curvature of the liquid in capillary pores, the chemical potential or the vapour pressure of the water is lowered compared with that of bulk pure water. The smaller the capillary and the thinner the layer of adsorbed water, the lower the water chemical potential. When freezing is initiated at 0 °C, only the water that has chemical potential equal to free water solidifies whereas water in narrow pores, or affected by the solid surface and therefore having lower potential, remains unfrozen. As the temperature of the system is lowered further, the chemical potential of water, in the form of ice, is correspondingly decreased[4] creating a gradient that successively induces increments of the unfrozen water to solidify. Thus, two condensed phases of water can coexist at temperatures below 0 °C—that is, unfrozen liquid water and ice crystals. The unfrozen water can be present either in narrow capillaries or as thin adsorbed layers. At each temperature the chemical potential of the unfrozen water is equal to that of ice at this temperature, being in thermodynamic equilibrium and physical juxtaposition with the ice. The relationship of thickness of unfrozen water to temperature can therefore be viewed as dependent on (and also a measure of) the change in chemical potential of water with the radius of pores or the distance from the solid surface.

The mode of change in chemical potential of a capillary condensate with pore size is well established; the Kelvin equation is a special case applicable to cylindrical capillaries. In general, the chemical potential depends on the nature of the liquid (its molar volume and surface tension) and is independent of the nature of the solid composing the walls of the pores. For cylindrical and slit-type capillaries it is proportional to r_h^{-1}. For thin adsorbed layers it was shown for nitrogen[6,7] and for water[8], that adsorption data relating some measure of the chemical potential (vapour pressure) to the thickness of adsorbed layer for many solid adsorbents fall on a common curve termed by de-Boer and his associates[6], the standard t curve. Thus the t curve seems to be characteristic of the adsorbate or adsorptive but virtually independent of the adsorbent. From these standard t curves it is also found that the chemical potential of the adsorbate usually varies with d^{-3} as expected for the classical van der Waals interaction resulting purely from dispersion forces acting between an atom and a slab[9]. Accordingly, and in view of the previous discussion on the mode of near-surface water freezing, we suggest that many porous bodies and adsorbents may have a common freezing curve relating the layer thickness of unfrozen water or the hydraulic radius of pores containing them, to temperatures below 0 °C. Our data show that this is the case for a variety of minerals (Fig. 1). From the data of Mazur it seems also to be the case for biological systems.

One puzzling and unresolved finding is that the coefficient in equations (1) and (2) relating the temperature below freezing point to the hydraulic radius or the equilibrium thickness of water on the surface (or when converted to other units,relating the chemical potential to the distance from the surface) is smaller by a factor of five to ten than that predicted from the Kelvin equation. The tentative explanation of Mazur[1] that the contact angle of the water in the pores is not 0 ° but 80 ° does not seem to be sufficient in view of the fact that three different adsorbents are involved in deriving the relationship given in equation (2). Also, the fact that even at every close proximity to the solid the interaction still seems to vary with d^{-1} rather than with d^{-3}, is puzzling. Thus none of the presently known modes of interaction between solid porous adsorbent and liquid adsorbate seems to account completely and quantitatively for these experimentally determined generalised freezing relationships.

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Received October 24, 1974; accepted March 26, 1975.

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Corrigenda

In the article "Evidence that mitogenic lectins induce changes in membrane fluidity" by R. E. Barnett, R. E. Scott, L. T. Furcht and J. H. Kersey (*Nature*, **249**, 465; 1974) the authors request that the following changes be made to the method. "The organisation of the membrane lipids was probed with a mixture of 3 spin labels, methyl 6-(4′,4′-dimethyloxazolidinyl-N-oxyl)-heptadecanoate (10%), N-(1,1-dimethyl-2-hydroxethyl)-6-(4′,4′-dimethyloxazolidinyl-N-oxyl)heptadecamide (30%), and 1-[2-(1,1-dimethyl-2-hydroxethylimino)cyclopentyl]-1-(4′,4′-dimethyloxazolidinyl-N-oxyl)undecane (60%). The observed differences in the order parameter could also be caused by differential uptake or metabolism of the labels by the cells."

In the article "Role of f-gravity in cosmological models" by C. Sivaram, K. P. Sinha and E. A. Lord (*Nature*, **249**, 640; 1974) equation (2) should have read

$$\dot{R}^2 = (\tfrac{8}{3})\pi G\rho R^2 + (\tfrac{2}{3})\Lambda c^2 R^2$$

The Λ-term occurs with a *positive* sign, so that a minimum in the function $R(t)$ will occur only if the coupling constant G_f is *negative* (and Λ_f positive). Thus the conclusions of this paper are valid only if the coupling constant of strong gravity is taken to be negative. The authors wish to thank J. V. Narlikar for pointing this anomaly out to them.

reviews

Is all that is not verse just prose?

P. N. Johnson-Laird

The Psychology of Language: An Introduction to Psycholinguistics and Generative Grammar. By J. A. Fodor, T. G. Bever and M. F. Garrett. (Mc-Graw-Hill Series in Psychology.) Pp. xvii+537. (McGraw-Hill: New York and London, 1974.) £8.80.

MOLIERE'S hero, Monsieur Jourdain, was amazed to learn that he had been speaking prose for 40 years. Is it really true that he spoke not just prose, but deep structures and transformations? Knowledge is a fine thing, indeed; but the question prompts another more serious one. How could it be shown that transformational grammars are wrong in principle?

One approach would be to establish the existence of certain sentences that they could not properly characterise. But grammatical transformations are an exceedingly powerful computational device, and it is very doubtful whether there are any such sentences. Another approach would be to show that no formal device could specify what sequences of words are grammatical and what sequences are ungrammatical. But even if this thesis were to be proved, which is extremely unlikely, it would establish too much. It would show that language was not governed by any determinate set of rules. Perhaps the general idea of transformational grammar is empirically invulnerable, and it remains only to establish the best grammar within its confines—a view which seems to be held by its current practitioners. There is, however, one aspect of the general theory that may be potentially testable. Transformational grammar should provide the basis for a psychology of language. It is this thesis, initially advanced by Chomsky, that Fodor, Bever and Garrett have set out to explore. Their original disciplines were, respectively, philosophy, linguistics, and psychology; and as one might expect from so talented an interdisciplinary team they have produced a comprehensive and thoughtful book.

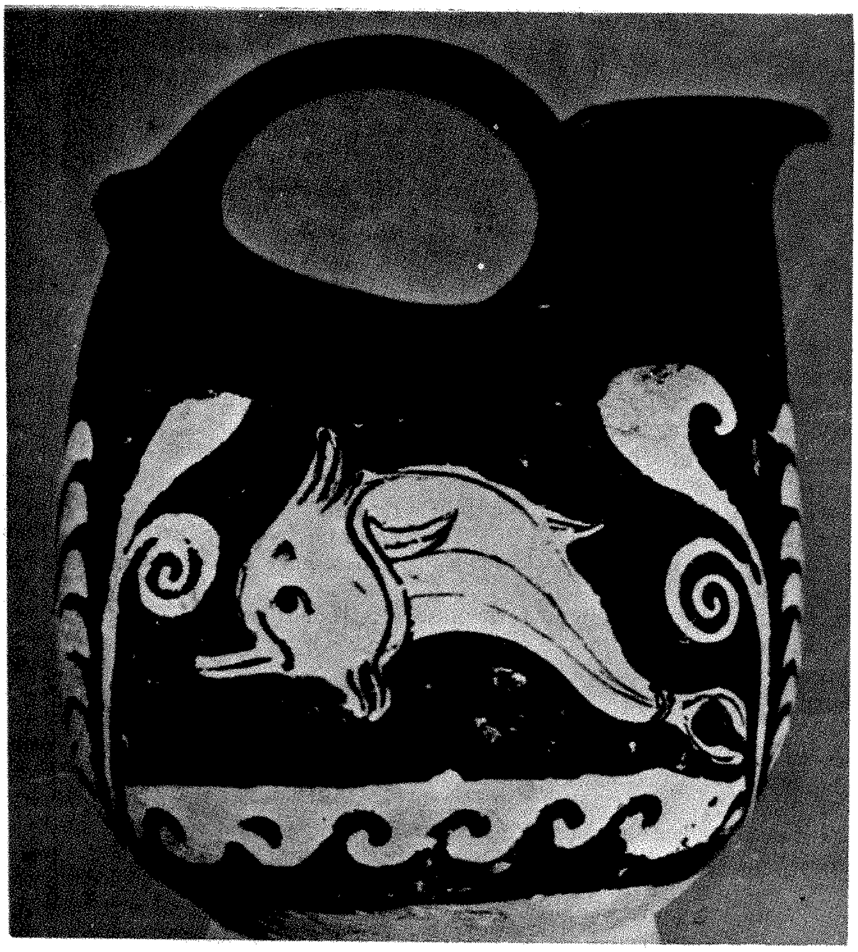
It is not difficult to appreciate why transformational grammar has proved so popular with psycholinguists. The authors review the deficiencies of its linguistic predecessors, the taxonomic grammars of the structuralist tradition, and show how neatly these grammars matched the outdated psychological assumptions of neobehaviourism. As Chomsky was one of the first theorists to point out the linguistic deficiencies of taxonomic grammars, it is scarcely surprising that his alternative approach to language was so appealing. His key assumption was that each sentence is derived by a series of transformations from an underlying deep structure specifying its fundamental grammatical relations. Later, of course, he came to believe that there is an interpretative component that also operates on the deep structure of a sentence in order to yield its essential meaning. So the crucial empirical test is whether it is possible to find traces of such structures and processes in a person's production and understanding of sentences. After one early inspirational article in *Psychology Today* (the American version), Chomsky was quick to close this empirical loophole. He invoked his notorious distinction between a speaker's knowledge or linguistic competence and how he exercises this knowledge in actual linguistic performance. The grammar is intended to apply only to linguistic competence—an assumption that has proved baffling to the point of apoplexy for those psychologists working within the Empiricist tradition.

A theory of linguistic performance might, however, incorporate a theory of competence couched in the form of a transformational grammar. Fodor, Bever and Garrett review the psycholinguistic literature in order to evaluate this proposition. They consider in detail studies of the production, perception and memory of sentences, naturally concentrating on the work that they and their colleagues have carried out at MIT. The moral they draw from their review is simple, but equivocal. There is evidence for the psychological reality of the notion of deep structure; there is little evidence for the psychological reality of processes corresponding to grammatical transformations. The authors draw a similar moral from their survey of studies of language acquisition. There is little evidence that children actually acquire grammatical transformations; there is, however, evidence that the order in which they master different sorts of sentence depends upon how difficult it is to relate them to their corresponding deep structures.

What an extraordinary state of affairs. It is as though each act of a play ended with a tableau appropriate, say, to *King Lear* although it had no discernable plot (or else it turned out to be that of *Charley's Aunt*). This is a problem that the playwright can resolve only by such time-honoured devices as, "with one bound the hero was free". Unfortunately, Fodor, Bever and Garrett have been unable to untie their hero; but there is perhaps an alternative explanation of the psychological phenomena. Indeed, looking closely at the text, one discovers such a possibility, which the authors have in their hands but let slip. They write (p. 270): " . . . so long as the deep structure is assumed to be that syntactic representation of a sentence which is semantically interpreted, hypotheses about the psychological effects of deep structure features tend to be confounded with hypotheses about the psychological effects of semantic relations". This confounding factor is the crux of the matter. One of the authors' typical experiments establishes that when a click is presented in the middle of a sentence, its apparent location migrates towards a boundary between clauses. The migration occurs even if there is no explicit boundary in the surface structure of the sentence. The authors accordingly argue that deep structure clauses have a perceptual integrity that leads to the relocation of events which interrupt them. Yet the crucial factor could be, not the coherence of a clauses's underlying syntactic relationships, but the coherence of its meaning. Transformational grammar is, however, by no means the only grammatical theory to assume that clauses have a meaning: proponents of Halliday's systemic grammar, for example, may be equally heartened by the conclusion that the semantic denouement is more important that the syntactic staging.

The main weakness of the book is the result of the authors' reluctance to look over the fence into their neighbour's backyard. They do not consider any of the current alternatives to transformational grammar and, in particular, the approach to language developed by the artificial intelligentsia working with computers. One suspects that they made a deliberate editorial decision to exclude this work—a great pity because many of the issues that they fail to resolve look more than susceptible to a procedural approach. In most other respects, however, the book is successful. Again and again, one is impressed by the authors' ability to think beyond an 'obvious' approach to a topic. They take a discerning route through the literature; and it is a real intellectual pleasure to follow in their footsteps.

The book is sophisticated but accessible. It is the definitive defence of the relevance of Chomsky's work to psycholinguistics, unlikely to be surpassed in its scope or thoroughness. Yet the defence fails. At the one point at which the general theory of transformational grammar is empirically vulnerable, it is all too vulnerable. The book probably marks the end of an era. It is unlikely that anyone will ever again mount so comprehensive a defence of transformational psycholinguistics. Monsieur Jourdain may go back to speaking simple prose. □



A Greek dolphin ceramic from the fourth century BC. From *Dolphins (The Undersea Discoveries of Jacques-Yves Cousteau)*. By Jacques-Yves Cousteau and Philippe Diolé. Translated by J. F. Bernard. Pp. 304+105 colour photographs. (Cassell: London, May 22, 1975.) £5.00.

Slipper animalcule

Paramecium : A Current Survey. Edited by W. J. Van Wagtendonk. Pp. xviii+499. (Elsevier Scientific: Amsterdam, London and New York, 1974.) $53.90.

THE genus *Paramecium* is familiar to most biologists and to many other scientists because it occupies a place in most courses on elementary biology. The amount of attention that has been paid to it in biological research is reflected in the fact that it has been the subject of more than 4,000 scientific papers and 4 monographs, the most recent of which is this volume edited by Professor Van Wagtendonk.

The book contains eight chapters contributed by workers actively engaged in research using *Paramecium*, and is said to provide "an up-to-date evaluation of research on all aspects of *Paramecium* biology." The eight subjects covered are: morphology, taxonomy and general biology; mating types; surface antigens; behaviour and motor responses; growth patterns and morphogenesis; structure; nutrition; and intracellular particles. Most chapters refer to the literature published up until 1969 or early 1970, though all are provided with addenda in which reference is made to work of the following two or three years. There is also a comprehensive bibliography of publications on *Paramecium* since 1953, the date of an earlier monograph on the subject by Wichterman.

The general introductory chapter by Vivier will be found the most useful by the average reader because it can be understood without much previous knowledge of the organism. This is not generally true of the other chapters, several of which assume an extensive background knowledge about the organism and specific facets of its biology, to which one is not introduced elsewhere in this book; most readers will not find such chapters fully comprehensible without preliminary reading of earlier work. All of the authors have, however, given an interesting account of the new knowledge being discovered at the frontiers of research upon *Paramecium* and all of the chapters contain much useful information for the protozoologist and cell biologist engaged in research and teaching, which recommends this volume as a valuable work of reference.

The reader will not find here any extensive information on nuclear function, on general physiological activities such as ionic regulation, contractile vacuole function or respiration, or on a number of minor facets of research on *Paramecium*, so that the monograph is not a comprehensive work. In a chapter on nutrition one might expect to find an account of the types of food eaten and the manner of entry into the body; not just a very detailed survey of the chemical dietary requirements for the growth of the animal. The view that mating types correspond to sexual types (p. 68) is less satisfying than the view that every *Paramecium* is hermaphrodite, producing gametic nuclei of both sexes, with mating types acting as devices to encourage cross fertilisation, analogous to self sterility and similar mechanisms that ensure cross pollination in plants. In the magnificently illustrated chapter on structure, a conventional analytical approach would have more successfully presented the information than the attempted synthetic method by which the authors built up a view of the structure from the component molecules, macromolecules and organelles.

The book is illustrated with a large number of electron micrographs and many other figures of a uniformly high standard. Errors and omissions in the figures, legends and tables are few, but are irritating and probably could have been avoided, although there are commendably few spelling errors.

Michael A. Sleigh

Astrophysical standards

Astrophysical Formulae: A Compendium for the Physicist and Astrophysicist. By Kenneth R. Lang. xxvii+735. (Springer: Berlin and New York, 1974.) $78.80.

In view of the enormous range of topics at present covered by astrophysics, it might be thought that a book which "is meant to be a reference source for the fundamental formulae of astrophysics" from nucleosynthesis to astrometry, would automatically be doomed to failure; but Kenneth Lang's attempt succeeds to a remarkable extent. The subject is divided into seven topics, grouped into five chapters—continuum radiation; monochromatic radiation; gas processes; nuclear astrophysics and high energy particles; astrometry and cosmology—and each topic is developed from first principles. This treatment, and the inclusion of several figures and tables of physical data, may make the book useful to the non-astronomical physicist, as promised in the subtitle, although beyond the basic development of each section the bias becomes increasingly too astrophysical for general use.

The disadvantage of this approach is that the discussion of any particular type of astronomical object becomes scattered throughout the text: neutron stars thus occur in such contexts as 'cosmology' (collapse of massive body), 'nuclear astrophysics' (URCA process), 'gas processes' (equation of state) and 'high energy particles' (X-radiation accretion in binary systems); similarly, the table of pulsar properties appears within the chapter on continuum radiation where the formula for dispersion measure is derived. (The pages of this table, incidentally, are printed in the wrong order.)

The comprehensive, 47 page, index minimises this drawback, and also allows quick access to the 69 tables of physical and astronomical data, the latter ranging from the planetary satellites through globular clusters to radio galaxies and quasars. A listing of the tables and figures in the contents section would have been useful though none is given. There are the inevitable misprints, for example "NGC 1270" for 1275 on page 554, and "log M_v" instead of M_v in the stellar luminosity function (Fig. 43); and there are also some omissions: Saturn X is missing from the list of satellites and the eccentricity and inclination of Pluto's orbit are not given in Table 57.

By its very nature a book of this kind cannot include enough information for day-to-day research, and its price too is likely to make it appear predominantly on library shelves. There is no doubt, however, that it will be an important reference work for any astrophysicist requiring basic information in a field outside his own, not just because of the concise presentation and clear explanation of the results, but also because it presents nearly 2,000 references, which include original sources from as far back as 125 BC (Hipparchus). This book should for many years remain one of the standard reference works in astrophysics.

Nigel Henbest

Slime organisms

The Mycetozoans. By Lindsay S. Olive. Pp. x+293. (Academic: New York and London, January 1975.) $23.50; £11.30.

The slime molds, both cellular and acellular have become increasingly popular over the last few years. Yet I suspect that most experimental biologists have little appreciation of the wealth of forms that exist and of how many riches are waiting to be tapped. In this book, Lindsay Olive does two things. He gives an admirable summary of some of the experimental studies of *Dictyostelium* and *Physarum* and, more important, discusses all the lesser known forms that are related in various interesting ways.

This is a monograph of the Mycetozoa in the classical sense. It discusses the taxonomy, the phylogeny, the morphology (including modern electron microscopic cellular morphology), and, in many cases, the experimental biology of all the known slime molds. What is most extraordinary is that many of the unusual organisms, some of which become 'missing links' between major groups, have all been described by Lindsay Olive himself. One would have thought that all the main types of organisms had been discovered long ago, but in the tradition of Roland Thaxter, Lindsay Olive, often in collaboration with Carmen Stoianovitch, has described many new species and whole new groups of organisms that were quite unknown until a few years ago. Because of the radical nature of these forms, Olive has not followed the well worn position of the past that myxomycetes and cellular slime molds be considered fungi. The main reason for this tradition was that the organisms were discovered by mycologists. Even though Olive himself is a mycologist he has taken the global view that the slime organisms are more closely allied to the protozoa and has fearlessly called them all Mycetozoa. This fine book, therefore, has much to offer to the protozoologist, the mycologist, the cell biologist, and the developmentalist.

J. T. Bonner

Calcium and secretion

Calcium and the Secretory Process. By Ronald P. Rubin. Pp. xiii+189. (Plenum: New York and London, 1974.) $18.00.

The central importance of calcium in a number of secretory processes—especially those in which secretion occurs by exocytosis—is now well established and Dr Rubin has provided us with a useful survey of the present position.

His book is divided into an introduction and three main chapters. In the first he reviews in considerable detail evidence for a role of calcium in a variety of secretory systems. The information presented in this chapter is excellent but suffers somewhat from a lack of balance in approach. The classical model system for the study of calcium in neurosecretion, the neuromuscular junction, is discussed only cursorily whereas many pages are devoted to discussions of whether calcium participates in the secretion of, for instance, the thyroid and other glands. In spite of the existence of many good reviews on the more classical system, I am quite sure that before being subjected to a steeplechase of secretory phenomena the reader would have benefited from a clear explanation of the role of calcium in secretion from the neuromuscular junction. This would have led naturally to a full discussion of other neurosecretory systems including the adrenal medulla and neurohypophysis and would have provided a sound base for examining systems in which the role of calcium is less clear.

The section entitled 'The role of calcium in secretion' provides a critical survey of current hypotheses. Restricting himself more or less to the classical secretory systems Rubin discusses the various mechanisms that may underlie cadcium-dependent exocytosis. The reader cannot fail to be struck by the absence of good experimental evidence on the ultimate mechanism by which calcium effects exocytosis and it is a pity that Dr Rubin does not point more clearly to hopeful new lines of attack.

In the final chapter Rubin discusses the involvement of cyclic AMP in secretion. Although no clear answer emerges, this chapter will be useful to those interested in this vexed question.

Parts of this book are of undoubted interest and will be useful to students of secretion; but the volume as a whole is rather unsatisfactory and its treatment of secretion is neither complete enough nor rigorous enough to ensure that it will not date rapidly.

P. F. Baker

obituary

Max Gluckman, Nuffield Research Professor of Social Anthropology in the University of Manchester, died in Jerusalem on April 13 during the tenureship of a visiting professorship at the Hebrew University. He was 64.

Born in Johannesburg, Gluckman began a brilliant academic career at the University of Witwatersrand and went to Exeter College, Oxford in 1934. After being engaged in fieldwork in Zululand from 1936–38, he joined the staff of the Rhodes–Livingstone Institute of Northern Rhodesia. There he embarked on field research in Barotseland, later to form the basis of his researches in anthropological jurisprudence. As director of the Institute from 1941–47, he laid the foundations of what was eventually to become the Manchester School of Theory and Research in Social Anthropology. He expanded the Institute's publication department to reach both a lay and an academic readership, organising contributions from a wide range of central African social and anthropological problems. He accepted a lectureship at Oxford in 1947, but moved in 1949 to the University of Manchester, where the chair of social anthropology had been specially created for him. He was primarily interested in establishing a research school and began by making his department homebase for his Rhodes–Livingstone team. The main aims of his research workers was to investigate the stresses and strains of modernisation, as well as the tribal custom and law. By the late 1950s the department had extended its research interests to India and the Middle East. He planned extensive and still continuing programmes of collaboration in research and teaching with the Israeli universities, among whose first projects were field studies by Israeli students in Beduin communities. With his confidence and drive, he organised and dominated the celebrated Gluckman seminar. His circle of contacts and the respect conferred on him by foreign visitors were invaluable links with other fields and views, and his researches led to the publication of *The Judicial Process among the Barotse of Northern Rhodesia,* an analysis of the concept of the 'reasonable man' in African jurisprudence. As chairman of the Association of Social Anthropologists, he organised in 1963 the first Anglo–American conference on new trends in the discipline. Among many academic honours were election as a fellow of the British Academy in 1969, and as an honorary member of the American Academy of Sciences in 1970. Gluckman was a very controversial man and will be remembered for the thought and discussion which he will continue to provoke.

announcements

Award

The **Johann–Georg–Zimmerman Prize** has been awarded to **Peter Magee** for his accomplishments in cancer research.

Appointments

J. W. Murray has been appointed Professor of Geology and head of the department in the University of Exeter.

The title of Central Electricity Generating Board Professor of Corrosion Science has been conferred on **P. R. Swann.**

International meetings

June 5, **Yeasts in industry,** Birmingham (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

June 16–18, **Protein and membrane structure and function,** Honolulu (C. S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California at Irvine, Irvine, California 92664).

July 14–18, **Origins and achievements of the Greenwich Observatory 1675–1975,** London (The Secretary, The Organising Committee, 4th Joint IAU/IUHPS Symposium, National Maritime Museum, Greenwich, London SE10 9NF, UK).

July 20–26, **Continuous culture,** Oxford (The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

Miscellaneous

Moon mission. NASA is asking for proposals for flight experiments from prospective investigators interested in participating in the Lunar Polar Orbiter mission (June, 1980). The latter is capable of extending to the entire moon, orbital and surface science results obtained from previous lunar missions (especially Apollo), and may serve as a prototype for similar ones to Mars and Mercury. Further information (by June 15, 1975) from: Dr F. D. Martin, Program Manager, Advanced Programs and Technology, Lunar Programs Office, Code SM, NASA, Washington, DC 20546.

Person to Person

Oceanography supervisor. Employed graduate working in London wishing to study for a higher degree in the history of oceanography seeks University Supervisor (Anita McConnell, 11 Arbor Court, Lordship Road, London N16, UK).

Accommodation wanted. British professor, on sabbatical leave from Canada, seeks furnished accommodation somewhere in the UK for his family (10, 7, 4) for the academic year 1975–76. 4 bedrooms, plus study, good-sized garden and easy access to open country essential (Dr A. R. Pugh, Department of Romance Languages, University of New Brunswick, Fredericton, New Brunswick, Canada).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

nature *May 22, 1975*

No time to be squabbling

A ROUTINE day at the United Nations in New York? No, a session at the Congress of the World Meteorological Organisation, one of the UN's specialised agencies, held recently in Geneva. At the end of the debate, South Africa was suspended from WMO until it renounces its policy of racial discrimination. The voting was 66 in favour, 25 against and 14 abstentions. In the same session the congress voted 81–14 to invite to WMO meetings observers from National Liberation Movements recognised by the Organisation of African Unity or the League of Arab States. Thus do some international scientific organisations conduct themselves these days; already the Food and Agricultural Organisation, UNESCO and the World Health Organisation have taken comparable restrictive measures against South Africa, and the recent Israel affair still causes a good deal of embarrassment at UNESCO.

> **Mr Tsou-Ching-meng** (China) deplored the regime imposed by white racialists in South Africa. His delegation considered that the proposal to suspend South Africa from Membership was right and proper. He stressed the sympathy of the Chinese people towards those struggling against colonialism and racialism in South Africa.
>
> **Mr Degefu** (Ethiopia) expressed his delegation's condemnation of the South African policy against non-white Africans as well as of that country's occupation of Namibia. Despite repeated condemnations, South Africa had not renounced its policy of racial discrimination.
>
> **Mr Pojani** (Albania) announced his full support of the proposal to suspend the Membership of South Africa. He deplored the aid given by imperialistic powers which enabled the South African regimes to continue.
>
> **Mr Fonseca** (Portugal) stressed that the democratic country he represented had nothing in common with the Portugal of 1974, which had been a colonial and fascist one. His Government severely condemned all forms of oppression, discrimination and inhuman treatment. He regretted that none of the documents made any mention of the new Portugal.

The very existence of South Africa is, of course, an affront to a majority of nations—indeed many of those who voted to retain South Africa in membership spoke of their abhorrence of her racial policies. And the depth of feeling can never be fully appreciated by the unoppressed whites of Europe. Nonetheless these latter also have deep feelings about the nature of science and the need for its pursuit to be free from politicisation, especially of the amateurish and self-congratulatory type. It is to the credit of the International Council of Scientific Unions (ICSU) that it has thus far been able to stand clear of most of the political nonsenses—although this has been at the expense of China's membership, since she resolutely refuses, despite all invitations, to be alongside Taiwan in the same meetings. It is also to the credit of the UNESCO staff that what gets to the conference table in that assembly has so far generally been universally acceptable.

But the question is bound to be asked—is the international science scene becoming increasingly politicised these days?

Was it ever otherwise, retort some of the wiser folk in the business—it's just that these days the west can be outvoted every time, so the politics are not those favourable to western countries; remember how the west used to vote to exclude China. This is a sane historical point of view, and history teaches us why people's present attitudes are what they are. Historical perspective, however, also gives us the opportunity to learn from the past in order not to re-live it. And one thing that ought to have been learnt is that the isolation of scientific and other intellectual pursuits, particularly from bodies which claim to be representative, profits no-one and is a futile gesture in the face of strong regimes.

There is another reason why the politicisation of science is particularly inappropriate at present. If voting in United Nations bodies were regularly to divide broadly such that developing countries, with their built-in majority, were able to force the pace against the developed countries which supply the money, then it is not inconceivable that an issue could arise on which the developed world decided it had had enough and would keep its money to itself. As yet there is no sign of this, but UNESCO is bound to be particularly closely watched in the coming year with its new Director-General, Mr M'Bow from Senegal, representative of the shift of power towards the developing countries. He will need to pursue a diplomatic path, and will not be helped by divisive confrontation.

But lest it be said that we are defining divisiveness as that which comes from the developing world, it should be added that some of the initiatives of the developed world seem only capable of generating bad feelings. Massachusetts Institute of Technology, which is negotiating a development contract for water resources in Saudi Arabia, is, it is reported, threatening that any act of religious discrimination will cause the contract to be cancelled. Saudi Arabia can hardly welcome Jews with open arms; it surely behoves MIT to use restraint and not to trigger confrontation by insisting on sending them. □

Some progress on the law of the sea

An indication of the slowness of the rate of progress at Geneva in the Third UN Conference on the Law of the Sea is the fact that at least two more sessions are now envisaged; the next will be convened in a year's time at UN Headquarters in New York. Little likelihood is therefore seen of an acceptable convention being hammered out before the end of 1976 at the earliest and it could not possibly come into force for at least two years after that. Progress has definitely been made, however, not least with the proposals covering scientific research.

DELAY has its dangers and there is still a strong possibility of a complete collapse of the negotiations on the law of the sea, which would be disastrous for developed and developing countries alike. Appeals have once again been made for countries to resist the temptation to take unilateral action before agreement is reached, though the patience of some of them is wearing thin and each year is likely to see further arbitrary claims being placed on statute books by geographically advantaged countries, such as the Latin American States, the USA and Iceland, followed by Canada, Mexico and Norway. Fortunately most nations are, so far, showing definite signs of restraint in staking their claims.

One of the chief stumbling blocks to negotiation is the multiplicity of groupings. It is not a case of two groups of states, 'developing' and 'developed', with clear negotiating positions. There are the geographically-advantaged (Argentine, Brazil, Canada, Kenya, the UK, the USA, and so on) and the geographically disadvantaged (led by The Netherlands), the coastal and the land-locked, the shelf locked, the island states, the archipelagic states and so on, all with different needs, wishes and ideas; each grouping overlaps inextricably with the others. There is little doubt that time is on the side of the geographically advantaged states—the concept of the 'Common Heritage of all Mankind' has been severely mauled and is unlikely to survive, except in a very weak and hardly recognisable form; this is mainly because of the activities of geographically advantaged developing states, rather than developed ones, though Canada has been conspicuously present at the kill.

Negotiating should now start in earnest both in the inter-sessional period and at the next session, though the stated likelihood of a further session after New York "if needed" in summer 1976 will tend to discourage such a move. Three weeks before the end of the conference, however, the chairmen of the three committees were asked to prepare an informal single negotiating text to serve as a procedural device to provide a basis for negotiation. This appeared on the last day of the session and, following scenes of copy snatching from other delegations' pigeon holes, the delegates departed to reconsider and reformulate their positions in preparation for the next occasion. It is vitally important that countries accept this text as a basis for negotiation; if they do not, there is little hope to be seen for any agreement in the foreseeable future.

Countries are still tending to wait for the so-called negotiating phase and, as a result, statements in public often differ quite considerably from the views put forward by the same delegate informally outside a meeting. One of the most surprising positions is that of India, which has in the past benefited almost more than any other developing country from international marine scientific research projects such as the International Indian Ocean Expedition but has been one of the leaders (in public) of the hardliners against any compromise on scientific research in the oceans. There should, in India's view, be no scientific research without very close control by the coastal state or an international authority.

The right to carry out marine scientific research suffered so much from a number of strong statements both for and against that the Chairman of the US Delegation (which came in for praise for the flexibility of its approach to the conference) stated in his closing statement, "I am particularly dismayed by continuing attempts to place restrictions on the conduct of marine scientific research. Knowledge of the oceans is important to all of us. Good science is free science; it is not a commodity that can be packaged and purchased in predetermined quantities. The conference should concentrate on means to ensure that all will enjoy the fruits of science, not on means to restrict science for fear it will only benefit the few".

Shortly before the end of the conference, and it was almost as if this document had not been noticed by the USA (or perhaps the Chairman of the Delegation had finalised his statement by then), a most unlikely group of developing countries, led by Mexico, which country has shown throughout an excellent grasp and understanding of the problems involved, presented a highly skilled compromise document entitled Draft Articles on Marine Scientific Research. This document has avoided most of the pitfalls which have proved unacceptable to one faction or another and should provide a real basis for the relevant part of the convention. Certain of the articles are, however, likely to be used by the developing countries as negotiating elements for improved arrangements for Transfer of Technology, including training and education in the marine sciences.

One of the strengths of this text is the way in which the controversial matter of fundamental as against resource-related research, has been dealt with. The onus is now placed on the coastal state involved to decide under which category an outsider's research programme falls. No doubt something like 90% of marine research will fall in the resource-related category but rules for conduct of such research, with the full consent and participation of the coastal state, are also provided for.

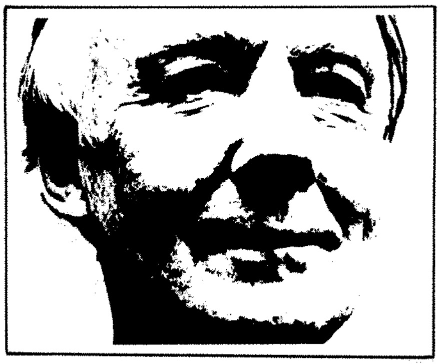
Unfortunately the author of the informal single negotiating text had failed to appreciate this approach and had gone back to some of the earlier, less informed proposed texts which call for the promotion "through competent international organisations of the establishment of criteria and guidelines concerning the differentiation between research directly related to the exploraion and exploitation of the living and non-living resources and fundamental research which is not directly related to exploration and exploitation of such resources"—a virtually impossible task. This text also still contains the fundamental error of linking exploration and exploitation, two completely different activities which should have separate regulations governing them. The former should be encouraged by all, in particular the developing countries, the latter clearly needs close supervision to ensure that any consequent profits are equitably distributed, that renewable resources are not destroyed in the process, that coasta states' rights are fully taken into account and so on.

The other bright spot in the conference has been the work done by an informal group chaired by Minister Jens Evensen of Norway which has developed a series of texts of draf articles on the 200-mile economic zone This group of senior delegates from about a third of the countries presen has had to deal with many diverse matters and interests, such as full use o fish stocks, size of allowable catches historical rights, access to resources by other countries and so on. These wil all be used as negotiating points in what will probably be the key to the convention, as most developing countries are more interested in immediate benefits which are seen to lie in their economic zones than more distant and less identifiable goals. No agreement on the economic zone will mean no convention. So far, however, the landlocked countries, which consist of two factions — those from Europe (like Switzerland and Austria) and the dozen or so from Africa—and the geographically disadvantaged states, consider that their interests have not yet been sufficiently taken into account.

Far more sinister are the activities proposed in the negotiating text for an International Seabed Authority. For instance, under a paragraph entitled "Contracts for Associated Operations", it says, "the Authority may enter into a contract, joint venture or any other such form of association, for the conduct of scientific research, or for the carrying out of a general survey or exploration of the Area . . . " It seems that under these plans the authority would have the right to deny access to the sea bed or to charge heavily for it. The structure proposed in the draft articles for the authority would be large and costly; in fact, it would virtually amount to the setting up of another new UN body which, in the present economic climate, many countries are determined to avoid. Certain of these countries would be prepared to see an authority formed provided it was self-supporting; that is, if its costs were covered by its income from contracts and exploitation licences. The most optimistic estimates are only for three or four contracts a year in the internatinal zone for the foreseeable future and this would certainly not maintain an authority of the size envisaged. Almost certainly the conference will soon start to look to the existing UN bodies, to see if one of them, such as the Intergovernmental Oceanographic Commission (IOC) or the Inter-Governmental Maritime Consultative Organisation (IMCO), could be expanded to take on additional tasks or if a new grouping could be formed.

It is clear from the Geneva manoeuvrings and deliberations that the chief interests of the developing countries lie, quite rightly, in the exploitation of living and mineral resources in the economic zone and in Transfer of Technology. Marine scientific research, instead of being called for urgently, is looked on with suspicion by countries who should know better and is being lumped with such matters as Coastal State Control of Ship-generated Pollution, the setting up of the International Sea-bed Authority for the International Zone, and numerous other matters of lesser interest which will be used as bargaining counters for immediate benefits. □

KENNETH MELLANBY



Weather vein

It is a common experience that when a subject which has previously caused us little concern comes to our serious notice, we seem to find ourselves hearing about it repeatedly. In my own recent experience, climatology is such a subject. In February this year I went to the island of La Gomera in the Canaries, to advise on possible agricultural and other developments. My remit was that developments should not adversely affect the ecology of the environment and should, if possible, encourage native flora and fauna.

La Gomera is nearly circular, some 12 miles in diameter, and it rises sharply to a height of nearly 6,000 feet in the middle. The highest land is covered by the remains of the original forest, most areas have been terraced and cultivated (though many farms have been abandoned) and the population is decreasing. For some years the weather has been unusually dry, and this February—at the end of what should have been the wet season, though little rain fell—the land was parched except where there was irrigation and the tanks, intended for use during the dry summer, were empty.

There are undoubtedly substantial water deposits on the island, but we do not know their capacity, nor how long they will be able to continue to supply fresh water before this is seriously contaminated with salt from the surrounding and underlying seawater. With a good rainfull, such as occurred fairly regularly until recently, the deposits were recharged.

A local consultant is optimistic, claiming that the sunspot cycle will ensure adequate rain in the next 18 months. Other experts are not so sanguine, however. To make a rational plan, climatological information is desperately needed. On it depends the future for the local farmers as much as that for those concerned with a very interesting and unusual development. La Gomera is only a tiny island, but it does epitomise a problem affecting vast areas in many continents, not least those of the Sahel area in adjacent Africa.

I had this example in mind when I attended a recent forum on "Neglected Research", where I heard how the pioneer work of Professor H. H. Lamb's Climatology Unit in the University of East Anglia had been completely denied support from any of the research councils. Yet when I was working on the water problems of La Gomera, I found that expert opinion, not only in Britain but also in other countries, always came back to the recognition of our current ignorance, and to the long-term importance of the work being tackled at Norwich by Lamb and his colleagues. It is difficult to understand why support is denied to a unit concerned with such an important and practical problem area, in which there is a good chance of some success.

It is generally believed that we in Britain have evolved the fairest and least corrupt method for sharing out research funds, even if it is so time-wasting. Many of our most eminent scientists sit day after day on the committees which make the recommendations on how grants should be given or withheld. This thankless task is carried out with care and, if possible, without undue bias. It would be cynical to suggest that those who give up so much of their time do so, partly, because this increases their chances of securing a substantial sum for their own work or for that of their department. It is true that we read in the minutes—"Professor Blank retired during the discussion of this item"—and then, surprise, surprise, follows the announcement that Blank's application for £50,000 has been successful. After all, Blank was put on the committee because of his expertise, and he would obviously not put forward a silly proposal! And occasionally a member's application is turned down. Yet the Lamb incident shows that our situation is not perfect. Even more serious, many unsuccessful and trivial research proposals do receive substantial support, and there is little to show for it.

In a field like research where chance plays so large a part, the very care with which we approach the problem may be a cause of its imperfections. I know, from my own experience, that one of the most substantial grants which led to marked research productivity was given on the whim of a minister and not on the advice of an expert committee. The trouble today is that such large sums of money are involved that we tend to build in complications which may waste much of that money. We forget that original research was once comparatively inexpensive. A plea for more small grants for unfashionable studies might produce unexpected results. □

international news

Soviet seminars are growing

from Vera Rich

Voronel: new development

THE Moscow Sunday seminars are more than a stop-gap for *refusnik* scientists awaiting a visa for Israel. According to their founder, Professor Aleksandr Voronel, who has recently been lecturing in the USA and Great Britain on the international implications of the seminars, they mark an important new development in Soviet science—the establishment of an "alternative" scientific community, which may well have considerable importance for world science as a whole.

When the original fifteen mathematicians and physicists met for the first such seminar in 1972, one of them, Professor Veniamin Levich, joked that it reminded him of his youth, to be back in a group who spoke of nothing but science. This, says Professor Voronel, pinpoints the position of "official" science in the Soviet Union, where, according to orthodox political thinking, "science cannot be non-Party, but must serve some political end". Although few scientists probably hold this view seriously, anyone who wants to go on quietly living and researching in the Soviet Union must at least subscribe to it formally, and, accordingly, meetings of official scientific bodies, from the Academy of Sciences downwards, cannot be conducted in a spirit of pure scientific enquiry, but will inevitably have some political overtones.

Accordingly, when, one by one, the *refusniks* were dismissed from their jobs, in spite of all their difficulties, they found themselves, for the first time in their professional careers, in the ideal state of "scientific detachment". Accordingly, the seminars began to attract not only would-be emigrants and Jews, but other scientists such as Yurii Orlov, Andrei Tverdokhlebov, Valentin Turchin, and Academician Andrei Sakharov. Some members of the group, although wishing to emigrate, have not, as yet, been dismissed from their posts. In spite of a certain turnover in the membership as some participants at last received their visas, the membership has steadily grown and, as at now includes more than 50 people, including 8 professors and 20 Candidates of Science (PhDs). In addition to the main Moscow group, there are members in several other cities who travel to Moscow when papers of particular interest to them are scheduled.

In spite of official attempts to crush the seminar (including, in Professor Voronel's opinion, the *volte face* of the emigration authorities in granting him an exit visa, and recent harassment of the leading members) it now exists as "a scientific community with a good tradition, independent of official organisations and of official policies". This, Voronel says, is a new and important development in Soviet Science, and he urges that western scientists should do all in their power to assist it.

Except in particular cases, such as psychiatry, in which scientific practice is distorted to accord with political theories and aims, he is opposed to any policy of a western boycott of Soviet science. A boycott, he says, may be "more high-principled", but is far less productive. It is not the scientists themselves who are responsible for Soviet science policy, but the officials, and the more contacts which can be established between western scientists and their Soviet colleagues, the better.

It is necessary to distinguish, howver, between what Professor Voronel calls "free" and "monopolised" contacts. A regular feature of almost every international congress or conference to which Soviet scientists are invited is the arrival, not of the expected delegate, but of some substitute appointed not on the grouds of academic merit but for reasons of political expediency. In such cases, Voronel's message is clear: accept no substitutes. If the expected delegate fails to arrive, then a boycott of his replacement's lecture will effectively stress the desire of western scientists for free exchange and their refusal to be dragooned by Soviet planners.

In so far as "free" contacts can be established, however, Professor Voronel is all in their favour. Although his attempt to hold an International Seminar in Moscow in 1974 was effectively broken up by the authorities, who arrested and hounded the leading members of the group and refused visas to the foreigners wishing to participate, contact between the seminar group and western scientists can still, he maintains, be accomplished. He suggests that any scientist visiting Moscow for an "official" conference, should, on a Sunday, make every possible effort to detach himself from the formal programme, and make his way to the seminar. Such a visit, he says, would not only be of interest to the scientists themselves, but would also be a significant statement in favour of academic freedom. Since, at present, Soviet policy favours detente and scientific cooperation (at the official level), the chances of a resolute scientist actually getting to the seminar should not be viewed too pessimistically. At the moment the authorities "have to be patient" with visiting scientists, and although, one presumes, the crucial time would be carefully filled with some other function or activity, he is convinced that, with determination, it should not prove impossible.

Meetings normally commence at noon, and details of venue and programme may be obtained from the seminar secretary, Professor Brailovskii. His address is Vernadskogo Prospekt 99, Block 1, Flat 128 (close to the Yugo-Zapadnaya metro station). Unfortunately, as part of the general policy of discouraging participation in the seminar, Professor Brailovskii's telephone was cut off some time ago.

Voronel stresses that such "free contacts" will not only benefit the *refusnik* members of the seminar, eager as they are to maintain an exchange of ideas with other scientists. Such meetings, he says, will benefit the whole concept of scientific exchange with the Soviet Union. All Soviet scientists, not only would-be emigrants, need contacts, scientific freedom, and the free exchange of ideas. within the framework of the whole Soviet scientific community, the seminar is important

Chemical plants on the doorstep

by Allan Piper

FOLLOWING the publication on May 12 of the report of the Court of Inquiry into last year's explosion at the Nypro chemical plant, the inhabitants of the villages around Flixborough are not alone in their misgivings over the report's assertion that "the siting of the works . . ." (in a sparsely populated area) " . . . was fortunate". Residents of the densely populated London boroughs of Ilford, Newham and Barking, who also live in the shadow of a chemical factory which formerly manufactured, and still stores, cyclohexane, are awaiting the outcome of an inquiry into a recent accident at the factory.

The accident, which resulted in the death of a 52-year-old worker, occurred on April 5, 1975, when the plant used in the manufacture of sorbitol exploded in the Ilford factory of Laporte Industries, a British-owned chemical company. The inquiry into the Laporte incident is the first to be ordered by the newly formed Health and Safety Commission whose involvement lends a wider interest to a continuing dispute over the siting of chemical plants that had already developed between the factory management and anxious local residents.

Laporte Industries moved to Ilford in 1961 after taking over the factory from a small pharmaceuticals company and since the move phthalic anhydride, cyclohexane, and sorbitol have, at various times, been manufactured there. Ever since the arrival of the company local people have been concerned about the standard of safety regulations enforced within the factory, claiming that the company has, until recently, acted less than openly with them. In spite of official assurances that everything possible is done to maintain satisfactory safety standards several incidents had occurred before the latest accident.

In 1969 part of the plant used to manufacture phthalic anhydride exploded. After the Flixborough disaster last June it became known that earlier the same year a small fire had broken out in the cyclohexane plant in Ilford. This was the first time that the residents became aware of the dangers posed by the manufacture of cyclohexane. Although the process was only being operated on a tenth of the scale of that at Flixborough, the Ilford factory is situated in a densely populated area and backs immediately on to a primary school of 650 pupils. Though cyclohexane and phthalic anhydride are no longer manufactured there, both chemicals are still stored there in large quantities.

Local concern led to the formation last year of a tenants' association, the Roding Environmental Safety Campaign (RESC), which is pressing Laporte to provide details of all hazardous processes operated at the factory and wants to see acceptable safety standards implemented at all plants.

But open days held at the factory last year were not reassuring. Neither is the RESC happy about the circumstances under which the cyclohexane plant was permanently shut down last October after a visit by the Factory Inspectorate. In their annual shareholders report, however, Laporte maintain that the closure was purely in deference to the understandable apprehension of local residents and was unconnected with the visit.

The worker who died in April was severely splashed with caustic potash solution following an explosion in a hydrogen plant used in the manufacture of sorbitol. The Factory Inspectorate are said to have visited plant associated with the hydrogen plant only a few weeks before the accident. Furthermore, the RESC claim to have understood from the company that sorbitol was a "safe" chemical and were not informed that part of the manufacturing process involved some risk.

The RESC has already pressed Parliament for a public enquiry into the running of the factory and a consideration of the siting of dangerous processes in the area. On April 11 Dennis Howell, the Minister of State for the Department of the Environment, expressed a willingness to help but on the question of siting he pointed out that "the Laporte factory was there before the houses and the school were erected, and not the other way round".

The RESC have at least obtained some backing from Redbridge Council who said earlier this month that they would continue to press Michael Foot, the Minister responsible, for a public enquiry into the safety of the works. In the same statement they also referred specifically to enquiries being conducted into the recent accident and said that the Factory Inspectorate had agreed to meet both the Council and the RESC to discuss the inquiry report following its publication.

Whether or not such discussions ever take place will depend entirely upon the Health and Safety Commission: the commission has stated that it would be left to them to "decide whether or not a report is to be published". Though officially they may not allow such a decision to be swayed by matters outside the incident under investigation it is unlikely that they will miss the opportunity to set a healthy precedent. □

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as a forum for scientific exchange independent of government and party policy.

The current atmosphere of détente lays considerable stress on scientific contact and exchange. The Soviet authorities, however, says Professor Voronel, wish to monopolise these contacts. Whether the recent flood of scientific and technological cooperation agreements which the Soviet Union has been signing will result in a genuine exchange of scientific opinions and information, not to the benefit of any one country, but to that of science as a whole rests, says Voronel, not with the politicians, but with the scientists. Now is the crucial time, he stresses, for western scientists to establish "new conditions and forms of contact" with their Soviet colleagues. □

Manpower planning and higher education

by Eleanor Lawrence

MANPOWER planning should have a greater part to play in decisions about the future expansion of British universities and polytechnics, according to Lord Crowther-Hunt, Minister of State overseeing higher education.

Speaking at a conference in London last week, he said that "at a time of economic and financial difficulty we need to be more than ever certain that the money we spend is well spent". He emphasised that "it simply will not do to allow universities and polytechnics to produce whatever people they fancy or to relate the number and kind of places they provide to the applicants that come forward". This was a passive approach which ignored policy variables which were in fact at the disposal of the government.

Undeterred by the admitted lack of success of previous efforts to forecast manpower demand, Lord Crowther-Hunt said that he wished to pursue a "positive approach to higher educated manpower, both to look again at what might be the needs of the economic system and to ask questions not simply about numbers of qualified people but the nature of the qualifications".

The Government could influence the situation in several ways, said Lord Crowther-Hunt. "At the one extreme, student grants and the counselling services of schools can surely affect the demand for places in higher education. At the other a considerable fraction of all graduates end up in public service. It follows that the size of the public sector and the qualifications demand in the public sector will themselves help to determine the number and kinds of graduates we need. It seems to me therefore that it is most unlikely that the best policy for a Government in the long term is simply to observe and to accept whatever is happening", he said.

- Hinting at further economies in the higher education system, Lord Crowther-Hunt dealt with the place of research in the universities in a speech to the Association of University Teachers in Manchester last Friday. About a quarter of university expenditure went into research, he said, which involved about one student in five.

"We all know of postgraduates in our universities who are neither contributing to the advance of worthwhile knowledge nor even adding in any worthwhile way to their qualifications", he said. "Does too much of our effort go into postgraduate work? Should there be a shift of emphasis to undergraduate education and teaching? Just as we have to become more efficient in industry, so do we in education". □

THE theme of this year's International Telecommunications Day, May 17, was "Telecommunications and Meteorology". This was also the theme of World Meteorological Day, March 23. Therein lies a story of real collaboration between two UN agencies which could set a pattern for the future.

Certainly the theme chosen is one that could hardly avoid involving both the agencies concerned—the International Telecommunications Union (ITU) and the World Meteorological Organisation (WMO). The important point is that it was chosen deliberately, the collaboration was intended and the celebrations planned jointly from the start. There are, it is true, a number of factors making it comparatively easy for these two agencies to work together. And each has been in existence long enough to be much more relaxed in its approach and, perhaps for that reason, rather more efficient in its operations than are its bigger brethren.

The trouble is that the large postwar agencies such as the FAO, UNESCO and the WHO tend to regard themselves as autonomous organisations advising—and at times seeming to pressurise—their member governments, rather than servicing them as do the ITU and the WMO. These big boys of the UN family have a certain arrogance that makes close collaboration difficult, not only among themselves but also with the smaller agencies. The latter feel swamped by the huge input that an agency such as UNESCO can allocate to a collaborative project or contribute to a meeting—and irritated by the way in which the same agency may take all the credit, even when the smaller, more expert body has done most of the planning and provided the greater part of the technical expertise.

The agencies do, of course, work well together on specific issues. But in dealing with wider issues—such as the problems relating to the world's water resources, which involves the FAO, UNESCO, the WMO, the WHO, the UN itself and now the United Nations Environment Programme (UNEP)—chaos still tends to reign. The apparently inevitable tendency of any new body to indulge in empire-building, of which the UNEP has in less than two years provided an already notorious example, makes matters worse.

In 1979 or 1980, one understands, it is planned to hold a major UN conference on the role of science in development. Is it too much to hope that, given four years in which to plan it, this may provide a real example of how UN agencies, almost every one of which is concerned in the application of science at one level or another, can collaborate for their own and everyone else's benefit? *from Peter Collins*

Plenty of potential for jojoba oil

by Colin Norman, Washington

IT sounds too good to be true. An unprepossessing desert shrub, now growing wild in parts of Mexico and the south-western United States, could, potentially, help save the sperm whale from extinction, rescue a number of Indian tribes from grinding poverty, and supply the automobile industry (among others) with a valuable raw material that is at present unavailable. The catch, though, is that it would take considerable capital investment and several years of effort to convert that potential into reality.

Nevertheless, a committee of the National Academy of Sciences last week enthusiastically urged the federal government to help underwrite the costs of cultivating the shrub on Indian lands. The committee suggested that plans should be set up immediately for establishing 400 acres of plantation a year over a five-year period.

Known as jojoba (pronounced 'hohoba'), the shrub produces peanut-sized seeds which contain about 50% by weight of a colourless, odourless oil. Its economic potential lies in the fact that the oil is unique among vegetable oils—it closely resembles sperm whale oil and when hydrogenated forms a hard wax which has a number of commercially promising features.

In 1971, the federal government banned use of sperm oil and other whale products in the United States because of the creature's threatened extinction. That move threw the automobile industry into a fit because sperm oil was considered essential for lubricating automatic transmissions. It has unique properties which enable it to withstand high pressures and temperatures,—and frustrating experiences with mineral oil substitutes which have been developed since 1971 attest to its value.

If jojoba turns out to be a good replacement for sperm oil, at the right price, it could rapidly be in high demand. Moreover, if the hydrogenated jojoba wax could be used as a replacement for spermaceti, which is also banned, or carnauba wax, which is now imported from Brazil at record prices, its economic potential would be even further enhanced.

To see whether the material is indeed as promising as people had been claiming, a research project directed by the University of Arizona's Office of Arid Lands Studies and the University of California at Riverside was begun in 1972. To start with, 87,000 pounds of jojoba seeds were harvested from wild shrubs by Indians in California and Arizona, and jojoba oil was farmed out to more than 75 corporations and research institutes for testing. It was the results of those tests, and of parallel tests carried out in Israel, which led to the enthusiasm displayed last week by the committee of the National Academy of Sciences.

The committee's report was intended as a general scientific survey of jojoba oil, and another committee is now taking a close look at the economics and the agricultural aspects of jojoba cultivation. Nevertheless, the report concluded that the evidence is sufficiently promising for the government to subsidise cultivation and an expanded research programme.

Chemical analysis of jojoba oil has confirmed that is it strikingly similar in composition to sperm oil. Both consist of wax esters, with similar chain lengths and an almost identical number of double bonds. And mechanical tests demonstrated that "jojoba oil can duplicate sperm oil performance as a high pressure lubricant".

As for the wax derived by hydrogenerating jojoba oil, the committee concluded that it has properties almost identical to those of carnauba wax. At present, carnauba wax, which is laboriously scraped from the fronds of palm trees, is imported into the United States entirely from Brazil. If hydrogenated jojoba oil could be developed into a domestic wax source, immune from the vagaries of international commerce, US industry would be assured of a continuous supply of uniform quality.

A key factor in the future for jojoba oil, however, is that the shrub could be grown on Indian reservations in the south-western United States, and it could provide a basis for some tribes to become economically self-sufficient. At present, many tribes in the area are living in conditions of extreme poverty.

The problem, however, is that it would be costly to establish plantations, and since it takes on average five years for the shrubs to start producing seeds, return on the investment would be slow. For those reasons, it is unlikely that industry will move rapidly into jojoba cultivation, and federal government funds will be needed.

According to experience in California, where 34 acres of jojoba were planted in 1974, start-up costs would be between $1,500 and $2,800 an acre. The most expensive part of the operation would be clearing and irrigating the land. But, once established, it would cost only about $100 an acre a year to maintain the plantation and to harvest the seeds. And, since jojoba bushes live for between 100 and 200 years, the initial investment would have ample time to pay off.

Studies have shown that cultivated jojoba bushes would produce, on average, 5 pounds of seeds, and a plantation would yield between 1,000 and 2,000 pounds of oil a year. Since sperm oil sells at about 40 cents a pound in those countries which still allow it to be marketed, and carnauba wax retails at about $2 a pound, the committee notes that " it can be seen that returns per acre from established jojoba plantations would be substantial."

Is it possible to predict with certainty that the oil would be in high demand if it were produced? The strict answer is no, but industry has so far shown sufficient interest to justify a qualified yes. Dr Demitrious M. Yermanos, Professor of Agronomy at the University of California, Riverside and a member of the academy committee, noted last week, for example, that jojoba oil has never been available in sufficient quantity for it to be market-tested. But he predicted that once it becomes available in quantity, industry will be very keen to use it. ☐

correspondence

Naples Zoological Station

Sɪʀ,—Some time ago (January 18, 1974) you published an interesting note on the Naples Zoological Station, which expressed the concern of the biological community about the future of the station and considered the importance of its future leadership as well as the question of maintaining international participation in its life and activities.

Since then there have been several changes and we should like to tell you how things stand at present.

After the increase of the annual budgetary support by the Italian government, the new set of statutes has been implemented. Consequently, we have seen the end of the seven-year-long "provisional Administration" and the formation of a new Council of Administration and an Executive Committee. A seat on the new Council of Administration is open to any country or institute that contributes more than £40,000 a year; such participation is seen as one of the most effective ways of strengthening and making more formal the international nature of the scientific collaboration that has been a main feature of the station for more than 100 years. We should very much like to think that Britain will have a seat on the new council.

To complete the new organisation of the station, we now need to appoint a Scientific Council (four members of which must be chosen from among foreign biologists, and one of these four by the International Union of Biological Sciences), and a director. It is hoped that these appointments will be made shortly.

Yours faithfully,

A. Bᴀʀʟᴀᴀᴍ

Naples Zoological Station

Leprosy

Sɪʀ, — Most voluntary agencies in Europe engaged in leprosy work are members of the International Federation of anti-leprosy Associations (ILEP) which brings together 22 member-organisations, of which 18 are European. The field of action of the federation extends to some 600 centres or projects in 75 countries afflicted with leprosy. According to the last statistical data available (1973), the federation is helping 1,106,560 leprosy patients, thus representing an important section of the voluntary agencies engaged in the world-wide anti-leprosy campaign. Among all the leprosy patients under treatment in the world, one out of three is aided by the federation. 96% receive domiciliary treatment and only 4% institutional care, as leprosy control is the top priority of the federation, in accordance with the WHO's policy. The total financial help provided by the member organisations is more than £4 million per year. More than 5% of the total budget is spent on research in leprosy (about £360,000 in 1974).

I hope you may consider this as an answer to the following sentence appearing in an article published recently (*Nature*, March 20): "Voluntary agencies in Europe collect more than £2 million annually for leprosy work, but most is still spent on institutional care".

Yours faithfully,

P. Vᴀɴ ᴅᴇɴ Wɪᴊɴɢᴀᴇʀᴛ

ILEP, 8000 Amiens,
France

A hundred years ago

The subject is treated of under three headings: General Dietetics, Special Dietetics of Health, and Dietetics in Sickness. The author commences with the question—What is the natural food of man? Flesh-eating animals have teeth, jaws, and limbs suitable for capture and tearing, vegetable feeders have bulky viscera, and so on. Applying similar arguments to the human race, "to judge by form and structure alone, the natural food of an adult man must be pronounced to be *nothing;*" from which we must necessarily deduce, as is indicated by other considerations, that man as man assumed his special characters *after* he commenced the employment of instruments for offence and defence. In fact, the developed heel, with which is correlated the non-arboreal habit, is incompatible with the naturally defenceless condition of our species.—from a review of Dr Chambers 'Manual of Diet'.

from *Nature*, **12**, 64; May 27, 1875.

news and views

Early man in the New World

from G. E. Kennedy

THE question of man's antiquity in the New World has been the source of long and often acrimonious debate. The matter has polarised around two widely differing points of view. An ultra-conservative approach, first developed by Holmes in the 1890s and brought to full culmination by Hrdlicka in this century, was based on two premises: that man was a relatively recent immigrant to the New World and that all Amerindians were of Mongoloid racial type. Hrdlicka was especially effective in destroying any claim to an early arrival of man in the New World and the vigour of his opposition had an almost paralytic effect on the early development of Palaeoindian studies. The conservative viewpoint, scepticism now tempered with careful scholarship, has continued in the work of Haynes, Irwin and others who maintain that empirical evidence of man in the New World prior to about 12,000 BP is very tenuous. The alternate viewpoint accepts considerable antiquity for man in the Western Hemisphere. Workers such as Wormington, MacNeish and Krieger, for example, while properly maintaining that many claims of great antiquity are spurious, have pointed to some early dates which are, in fact, very difficult to dispute.

The evidence of man's occupation of the New World prior to about 12,000 BP falls neatly into two categories: archaeological evidence and human skeletal evidence. Unfortunately, the association of tools and human remains occurs at very few early sites. The Yuha site in southern California, dated at 21,500 BP ∓2,000 (Childers, *Anthrop. J. Can.*, **12**, 2; 1974) and the Marmes Rock Shelter, in Washington state, dated to about 13,000 BP (*Fryxell et al.*, *Am. Ant.*, **33**, 511; 1968) are two of the very few early sites where such associations occur.

George Carter, whose archaeological work has centred mainly in southern California, is one of the leading proponents of great antiquity for man in the New World; he has suggested that stone tools from the Texas Street site, in San Diego, for example, possibly date to the last interglacial (*Pleistocene Man at San Diego*, Baltimore, 1957). His claims have been widely disputed and most archaeologists consider that the Texas Street 'tools' were formed by natural rather than human processes. The Calico archaeological site, in the desert east of Los Angeles, has been claimed to demonstrate evidence of human occupation at about 50,000 to 80,000 years ago and dates of 120,000 years ago, based on geological evidence, have also been suggested (Leakey, Simpson and Clements, *Science*, **160**, 1022; 1968). Evidence has been put forward at Calico to support the existence of one and perhaps several hearths deeply buried in an alluvial fan. Though the human origin of the 'hearths' has been questioned the interpretation, as tools, of flaked and chipped lithic material from the site is very difficult to dispute. Conclusions regarding the Calico site should await a more comprehensive study of the lithic materials than has been undertaken so far. Other early sites, from Canada to South America, show artefacts of unquestioned human design in firmly dated contexts. The Lewisville site, in eastern Texas, comprises more than 21 hearths which, in many cases, contain the burnt remains of now-extinct Upper Pleistocene fauna; charcoal from two of these hearths has been dated to more than 37,000 years (Crook and Harris, *Am. Ant.*, **23**, 233; 1958). On Santa Rosa Island, off the southern California coast, burnt mammoth bone has been dated to 29,700∓3,000 (L290R, Broecker and Kulp, *Science*, **126**, 1324; 1957). Other material, obtained from a fire area on the island was reportedly beyond the range of radiocarbon dating (UCLA 749, Berger and Libby, *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1678; 1966). A recently reported site from the Old Crow River basin in north-eastern Canada has yielded a worked caribou tibia dated to $27,000^{+3,000}_{-2,000}$ (Irving and Harington, *Science*, **179**, 335; 1973). One of the most indisputable of the palaeoindian tool sites is in the Ayacucho Valley of Peru; here, in a deposit underlying a level with a radiocarbon date of 19,600 BP ∓3,000, MacNeish has found stone tools in association with the bones of Pleistocene fauna (*Scient. Am.*, **224**, 36; 1971). On the basis of the early occurence of this "core tool tradition" in South America, MacNeish has suggested that man may have entered the New World as early as 100,000 years ago.

In the analysis of human skeletal material, the field of Amerindian palaeontology is still in its infancy, in spite of nineteenth century origins. Very little early human material is known and of that material provenance and associations are sometimes unsure. With a few notable exceptions none of the very early material has been completely described and without exception none of this early material has been analysed within the statistical framework of population theory. Such treatment, in a comparative context, is vital if we are to gain objective insight into the origins and relationships of the earliest Amerindians.

Of the human material that is available, the child's remains from Taber, Alberta may have present claim to the greatest antiquity; stratigraphical evidence suggests that the site is more than 37,000 years old and may be as much as 60,000 years old (A. Stalker, *Am. Ant.*, **34**, 425; 1969). The racemisation dating technique has been of considerable importance in placing many specimens, long undated, in a chronological framework. The earliest of the racemisation dates has been obtained from the 'Del Mar' skull; this nearly complete cranium from southern California has been dated to 48,000 BP (Bada *et al.*, *Science*, **184**, 791; 1974). A frontal fragment from the same area has been dated to 44,000 BP and a potentially important group of post-cranial material has been dated at 28,000 BP (*ibid.*). This latter material, however, recovered 49 years ago, has not yet been cleared from its matrix. The 'Los Angeles Man' cranium has been dated with both radiocarbon and racemisation and these dates are in close agreement: >23,600 in the first case (Berger *et al.*, *Contr. Arch. Res Fac.*, No 12, University of California at Berkeley, 1971) and 26,000 in the latter (Bada *et al.*, *ibid.*). The clear antiquity of Los Angeles Man makes the unaccountable loss of the associated post-cranial material very regrettable. The Laguna skull, also from the southern California coastal region, has been dated to 17,150∓1,470

(Berger *et al.*, *ibid.*).

Although there is no question of the Mongoloid affinities of the modern Amerindians the origins of the earliest groups are not as clear. Some anthropologists consider that the Mongoloid populations of Asia are among the most recently developed of the major human racial groups. Coon and Birdsell, for example, would argue that the Mongoloids developed, as a population, not more than 10,000 years ago. If this is true, then obviously the earlier New World immigrants must have had non-Mongoloid affinities. Birdsell, Hooton, Howells and others have, in fact, detected possible Caucasoid elements in the remains of some early Amerindians and have linked these with Upper Pleistocene populations in eastern Asia; Birdsell has used the term 'Amurian' for these Asian groups. The question of the population affinities of the earliest Amerindians is a complex one and the answer must await further data from both Asia and the New World.

Oddballs and galaxy formation

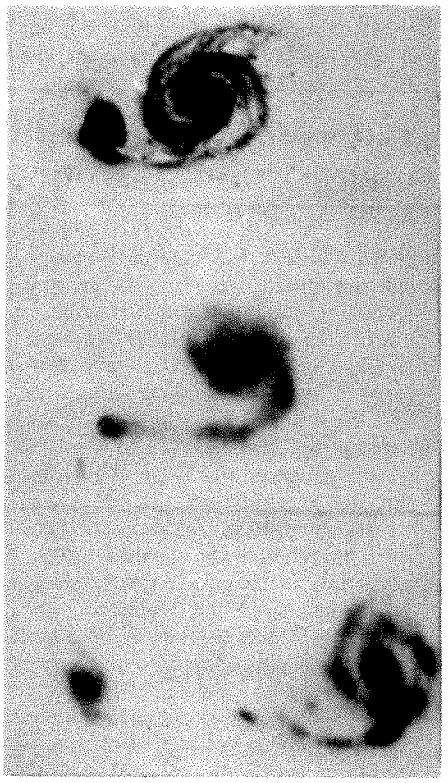
from James Binney

THERE seems to be no limit to the number of weird objects one can turn up in the sky. We now have catalogues compiled by Zwicky, Vorontsov-Velyaminov and Arp, containing over ten thousand peculiar extragalactic objects. These range from almost stellar, highly luminous emission line objects to groups of distorted, apparently interacting galaxies. Many of the objects identified as peculiar on optical plates turn up also as unusually strong emitters in other ranges of the electromagnetic spectrum. Undoubtedly these objects would well reward greater study. Indeed three recent papers suggest that they may be able to teach us something about one of the greatest enigmas of modern cosmology: the formation of galaxies.

Freeman and de Vaucouleurs (*Astrophys. J.*, **194**, 569; 1974) suggest that pairs of an irregular galaxy and a spheroidal galaxy could be produced by the collision of spiral galaxies with an hypothesised intergalactic cloud of neutral hydrogen (see *Nature*, **254**, 181; 1975). Freeman and de Vaucouleurs suggest that the Universe may be filled, not necessarily uniformly, by a few hundred clouds of neutral hydrogen per massive galaxy. If the radii and masses of these clouds lie in a narrow region around 10^5 light-years and 10^9 solar masses they claim one can account for the observed density of spheroidal–irregular galaxy pairs in terms of the compression and fragmentation of the gas involved in the collision of the gaseous part of a spiral galaxy with one of these clouds. Stars formed from this gas would form the irregular or ring galaxy while the stellar component of the spiral galaxy would pass on as a spheroidal system.

This links with the work of Oemler who has concluded from a study of 15 rich clusters of galaxies (*Astrophys. J.*, **194**, 1; 1974) that there are three basic types of rich clusters: spherical ones, like the cluster Abell 2199, which contain an irregular giant galaxy at the centre and are rich in elliptical galaxies, clusters rich in lenticular galaxies and spiral rich clusters. Oemler's work tends to confirm the suggestion of Gunn and Gott (*Astrophys. J.*, **176**, 1; 1972) that the spiral rich clusters are ones in which the general cosmic expansion, though slowed by gravity, has not yet been reversed, whereas the clusters rich in lenticulars are ones where recollapse has occurred and a substantial fraction of the spiral galaxies initially present have been stripped of their disk component in interactions with an intergalactic medium. Could this intergalactic medium consist of the gas clouds of Freeman and de Vaucouleurs? Is it the mass of these clouds which binds the galaxies into a cluster in spite of the very high velocities these galaxies appear to possess?

Another quite different interpretation of some of these multiple systems of interacting peculiar galaxies is argued in a research note by Vorontsov-Velyaminov (*Astr. Astrophys.*, **37**, 425; 1974). Vorontsov-Velyaminov believes he has identified a number of systems caught actually in the act of breaking up into two or more parts, or 'gemmation' as he calls it. The figure for example is taken to show three stages in the ejecion of a satellite galaxy from a late-type spiral galaxy. Though this example could perhaps be more easily understood in terms of satellite capture for which numerical models by Toomre and others exist (*Astrophys. J.*, **178**, 623; 1972), others of Vorontsov-Velyaminov's specimens could well represent small groups of galaxies forming from a large cloud of hydrogen. A fragmentation process of this type has been suggested by Casini *et al.* (IAU meeting, Trieste, September 1974) in relation to Markarian's very similar groups of galaxies.



Proposed stages in ejection of a satellite galaxy from a late-type spiral galaxy; from Vorontsov-Velyaminov, *Astr. Astrophys.*, 37, 425; 1974.

All this tinkering with the population of dwarf galaxies does not of course directly help us to understand the more fundamental formation of giant galaxies like our own. Nothing daunted by the failure of cosmologists to come up with a satisfactory mechanism for triggering the formation of condensed objects in an expanding Universe, Meszaros (*Astr. Astrophys.*, **38**, 5; 1975) has explored the possibility that a primordial population of black holes of about solar mass might have precipitated the much later collapse of galactic masses. He finds that if the total mass of black holes in the present-day Universe were between one and ten times the mass contained in visible galaxies, the statistical fluctuations in the number density of black holes in the early Universe may have caused perturbations in the overall matter density of the Universe to grow from the end of the first 10^{-4}s of our history. These black holes would now be clustered around galaxies in enormous numbers (about 10^{12} around the Milky Way) forming a massive halo of the type whose existence has been suspected on other grounds (Ostriker and Peebles, *Astrophys. J.*, **186**, 467; 1973). Unfortunately the detection of these black holes is well nigh impossible and the physics underlying theories of their formation rather doubtful.

The real problem of galaxy formation remain very much unsolved. The greatest difficulty is that we still have no idea what induced the formation of the first bound objects in an expanding Universe. One suspects that the origin of these local inhomogeneities in the structure of the Universe will be understood only when we know why the Universe is so remarkably smooth and homogeneous on a large scale and a major effort is now going into the possibility that this isotropy is the result of quantum effects back in the period when the Compton wavelength of an elementary particle was comparable with the radius of the then visible Universe. The other urgent task in cosmology is to understand how in the more recent past great masses of gas collapsed to form galaxies and clusters of galaxies. This task is particularly exciting because there is every reason to believe that we should be able to actually see this happening by looking out into distant space. What we require are detailed models of the collapse to enable us to identify a nascent galaxy when we see one.

How to study the birth of galaxies using radio-astronomical techniques

by John Gribbin

If you accept the standard picture, galaxies and clusters of galaxies were formed by condensation from a more uniform distribution of hydrogen gas. But just how hydrogen clouds transformed into galaxies is far from clear, and there has seemed little chance of making the situation more clear by direct observation. Now, however, Sunyaev and Zel'dovich have come up with a suggestion that radio observations might provide a clue to events occurring at the epoch when galaxies were forming in the Universe (*Mon. Not. R. astr. Soc.*, **171,** 375–379; 1975).

Some of the principal questions which are far from being answered are listed by the two Soviet astronomers: Were globular clusters formed first and later joined into galaxies? Did the first step consist of separation and condensation of giant gas clouds (with masses in the range 10^{13}–10^{15} $M_\odot$) which later disintegrated into galaxies, globular clusters and individual stars? At what redshift z (relative to us) did this occur? And what were the density and temperature of the gas clouds from which galaxies formed?

In principle at least, some of these questions might be answered by radio-astronomy studies not hopelessly more sophisticated than those of the present day. One approach would be to search for the redshifted 21-cm hydrogen line emission from the epoch just before galaxies were formed.

Sunyaev and Zel'dovich consider the case of spherical galaxies with mass 3×10^{14} $M_\odot$ and diameter 2 Mpc, separated by 30 Mpc. Making reasonable assumptions about the proportion of neutral hydrogen, H I and He I, and taking the diameter of a cloud just before stars formed as also being typically 2 Mpc, it turns out that the hydrogen line should still be seen as an emission line, even with a redshift of 5–10, if at least half the protogalactic material was in the form of hot gas ($T\sim7{,}000$ K). The increase in temperature of the observed line over the background is, however, only expected to be some 7 K, for an observer co-moving with the protogalaxy.

But to an observer on Earth the line will be redshifted to 100–200 cm wavelength, a region where the overall background has a brightness temperature ranging from 20 to 200 K. Is it sensible to expect to be able to detect the redshifted hydrogen line above this background? The answer depends, of course, on just how many proto-galactic clouds there might be at these large redshifts in each solid angle of sky subtended by the observing telescope. Sunyaev and Zel'dovich conclude that no more than 200 protoclusters might be visible over the whole sky at any one wavelength, and that "observations with a variable wavelength are essential, both in order to augment the number of protoclouds observed and also to distinguish them from sources of different type with continuum spectra".

They also suggest still more complicated experiments, but this one test is likely to be enough to tax the ingenuity of radio astronomers at present. Still, the possible results would seem to merit some effort being made. As Sunyaev and Zel'dovich say, "these observations are very difficult and substantial improvement of existing equipment will be needed to fulfil the task, but the importance of the problem seems . . . to warrant discussion in order to attract the attention of experimentalists".

- **One in a million.** Meanwhile, a reminder to galaxy hunters that not all of the peculiarities of unusual astronomical objects result from physical processes hinting at new universal laws, and that coincidences do happen, has come from a study by de Vaucouleurs and de Vaucouleurs of NGC985—a ring galaxy with a Seyfert nucleus (*Astrophys. J. Lett.*, **197,** L1; 1975).

If ring galaxies do form when normal galaxies collide with intergalactic clouds (as Binney discusses above), and if, as seems likely, some (and perhaps all) normal galaxies experience Seyfert outbursts, it is plausible that the formation of the ring in NGC985 and the presence of Seyfert activity are completely unrelated. De Vaucouleurs and de Vaucouleurs suggest that the probability of a randomly chosen Sa–Sc spiral having encountered an intergalactic gas cloud in the past 5×10^7 yr (so that the ring is still visible) is about 10^{-4}, and that the probability that the nucleus of a giant spiral is seen today in the Seyfert stage is about 10^{-2}. That makes NGC985 literally a galaxy in a million.

Specific response of *Drosophila* cells to heat shock

from Benjamin Lewin

When larvae of *Drosophila melanogaster* are subjected to a heat shock, usually a rise in temperature to 37 °C from the usual 25 °C, a change in the pattern of puffing is seen in the salivary gland chromosomes; preexisting puffs regress and new puffs appear at several, specific sites. The same response is produced by treatment with respiratory inhibitors and occurs during recovery from anaerobiosis. The switch to puffing at these bands presumably represents the expression of new gene functions and, because of the different treatments which elicit it, may perhaps constitute some generalised response to certain environmental abuses of *Drosophila* larvae. The specific puffing pattern can be provoked by heat shock of isolated salivary glands and so must be autonomous. It is not known what functions it represents; but the limited number of bands that is involved and their coordinate expression in response to more than one treatment of larvae or excised tissues suggest that this may be a useful system for investigating genetic control and defining the number of functions in individual bands.

Two recent series of experiments exploit the remarkable observation that heat-shocked tissue culture cells of *Drosophila* seem to display the same response as whole larvae or isolated salivary glands. The cultured cells in both sets of experiments are derived from Schneider's S2 line, originally grown from mixed early embryonic cells, and therefore unfortunately not of well defined phenotype. But the cells' apparently autonomous response implies that the results of experiments with them may be representative of the *in vivo* gene expression of *Drosophila* larvae.

A study of the messenger RNA present in normally grown and heat-induced cultured cells was recently reported by Spradling, Penman and Pardue (*Cell*, **4**, 395–404, 1975). The poly(A)-containing mRNA of the polysomes of these cultured cells shows a very broad size distribution on sucrose gradients, similar to that seen with many cultured mammalian and insect cells. When this RNA is hybridised *in situ* with salivary gland chromosomes, it binds to many loci, apparently representing at least 50 bands on the four chromosomes; in addition there is intense binding at the chromocentre. Of course, it is not surprising that many genes in euchromatin should be expressed; some bands, about a dozen, are more heavily labelled than most and these are seen more clearly by using lower concentrations of the labelled mRNA. This reduction in concentration leaves the chromocentre still heavily labelled, however; the chromocentre seems to include two types of heterochromatin : α-heterochromatin which consists of satellite DNA and is under replicated in the formation of polytene chromosomes, and β-heterochromatin, about which less is known. Since satellite DNA seems to be inert in transcription, these results suggest that α-heterochromatin may contain genetic material that codes for mRNA. Another implication of these results is that a comparatively small number of bands is expressed in the cultured cells, certainly fewer than (say) a hundred. Yet estimates of complexity made from the reaction between mRNA and cDNA or isolated nonrepetitive DNA suggested the expression of a much larger number of genes, of the order of 2,000 (cited in Bishop *et al.*, *Nature*, **250**, 199–203; 1974) Kinetic analysis suggests that most messengers represent genes in nonrepetitive DNA; and genetic analysis suggests that each band specifies a single genetic function This discrepancy is therefore difficult to resolve at present if all the messenger species in the population are able to hybridise *in situ*

A drastic change occurs in the mRNA when the cultured cells are shifted to 37 °C. Within an hour, the broad distribution on a sucrose gradient is replaced by four distinct peaks, in a somewhat similar overall size range When the poly(A)-containing mRNA of the heat-shocked cells is hybridised *in situ* with salivary gland chromosomes, it reacts with only a small number of bands, those that are puffed during heat shock in the larva or excised tissue. This suggests that the same switch in gene expression occurs in the cultured cells as in the intact tissue. The most heavily labelled band is 87C1, and other labelled bands include 63C, 67B, 87A, 93D, 95D. Although very similar to the heat-shocked puff pattern, the hybridisation pattern is not indentical; some bands that puff in larvae, 33B, 64F, 70A, do not hybridise specifically with the heat-shocked mRNA, and band 85A binds mRNA after heat shock but does not puff in larvae. Whether this represents a genuine difference in gene expression between cultured cells and salivary glands or is rather a consequence of different assay systems is not known; however, the same repression of previously functioning bands and almost identical expression of new bands is produced by heat shock in both cases.

Changes in protein synthesis

A start towards identifying the functions of the heat-shock puffs was made last year by Tissieres, Mitchell and Tracy (*J. molec Biol.*, **84**, 389–398; 1974) when they reported that heat shock causes changes in the pattern of protein biosynthesis; synthesis of most proteins is much reduced but synthesis of a small number, about six, is induced. This provides an obvious correlation with the changes now observed in mRNA synthesis. Tissieres *et al.* observed that the principal induced protein has a size of about 70,000 daltons and corresponds to some 20% of the newly synthesised protein. Since the heat-shocked puff that they observed at 87B accumulated newly synthesised RNA the most intensely, they suggested that this protein may be coded by a gene in the 87B band.

Using the Schneider S2 culture line, Mckenzie, Henikoff and Meselson (*Proc. natn. Acad. Sci. U.S.A*, **72**, 1117–1121; 1975) now report that the same changes in protein synthesis, followed by SDS gel electrophoresis, are exhibited in response to heat shock After incubation at 37 °C for 50 min, the proteins induced in the heat-shocked cells comigrate with those induced by temperature shock of isolated salivary glands. The most prominent band again has a size of some 70,000 daltons.

A large shift in the polysome profile took place upon heat shock. At 25 °C, the polysome distribution shows a peak at 12–13 ribosomes per message; but after incubation at 37 °C for 10 min these polysomes disaggregate. After 30 min at the higher temperature, larger polysomes form, with 20–30 ribosomes on each messenger. At 50 min, this is the type of polysome observed on a gradient. Addition of actinomycin does not prevent the disaggregation but does inhibit appearance of the new (larger) peak, suggesting that these polysomes include the messengers newly synthesised in response to the heat shock. When the RNA from the heat-induced polysomes was isolated, it gave a single peak on a sucrose gradient, corresponding to a length of about 3,300 nucleotides. Presumably this peak includes several messenger species. This length of mRNA is sufficient to code for protein of up to some 120,000 daltons, and therefore can certainly be viewed as a possible messenger for the 70,000 dalton protein. When used for *in situ* hybridisation with salivary gland chromosomes, the RNA of the heat-shocked polysomes predominantly labelled band 87B of the third chromosome; however, in a note added in proof, McKenzie *et al.* conclude that this labelled material may in fact correspond to band 87C1. Bands 87A and 93D both also are labelled in this hybridisation, as is the chromocentre. The coincidence between the heavy synthesis of the 70,000 dalton protein and the heavy labelling of 87C1 lends support to the idea that this band may include the gene for this protein.

The identification of an individual band with a single protein, and the availability of the messenger RNA intermediate, in principle offer an opportunity to investigate in one instance the relationship between band and gene. Although genetic analysis suggests an equation between a band and a genetic function identifiable by mutation, which implies that only a small part of the band is used to code for mutationally visible protein, this does not formally exclude models postulating that other DNA in the band may code for proteins not identifiable by lethal mutations. The heat-shock system may offer a valuable independent opportunity to investigate the function of the band at the molecular level. If the functions of the proteins coded by the heat-shock puffs can be identified, this system also may allow the coordinate control of separated genetic functions to be studied since it comprises a small number of proteins whose synthesis readily can be switched on and off. And of course, it remains to be seen exactly how the expression of these functions enables the organism to withstand heat shock. □

Structure of a lectin

Crystallographic results

from C. C. F. Blake

ALTHOUGH the major effort in protein crystallography is at present centred on enzyme structure, structural studies on certain non-enzyme proteins may provide information of somewhat wider biological importance. One such group of proteins is the lectins. These molecules, found largely in plants, and particularly in seeds, have a number of interesting and unusual biological properties that derive from their ability to interact with cell surfaces. Their characteristic property is the ability to agglutinate erythrocytes, sometimes in a blood-group specific manner. Other cells may also be affected and most interestingly some lectins are preferentially able to agglutinate cells that have been transformed by viruses, carcinogens or radiation. In addition certain lectins are mitogenic; that is, they are able to stimulate the transformation of lymphocytes from small resting cells to large actively growing cells that may ultimately undergo mitotic division. The specific inhibition of the activity of many lectins by simple sugars has led to the conclusion that lectins bind specifically to saccharide chains on the surfaces of cells. The study of the structure and activity of these proteins could lead to an understanding of the molecular basis of their sugar specificity and the mechanism of their action on cells, which could in turn provide an insight into the structure and behaviour of the cells themselves.

Because of its availability and ease of extraction the most extensively studied lectin is concanavalin A from the jack bean. It is also one of the more interesting members of the group, because in addition to being haemagglutining, it is also mitogenic and capable of specifically agglutinating malignant cells. The concanavalin A molecule is composed of polypeptide chains that have a molecular weight of 25,500; below *p*H 6 the molecular species is a dimer, and above *p*H 6.5 it is a tetramer. The protein apparently binds to glucosyl and mannosyl residues at the non-reducing end of oligosaccharides, and it is most potently inhibited by α-methyl-D-manno- and glucopyranosides. Like other lectins concanavalin A requires the ordered binding of manganese and calcium ions before it can exhibit its sugar binding capacity.

Since concanavalin A was crystallised by J. B. Sumner as long ago as 1919, it is not surprising that it has more recently been the subject of extensive X-ray diffraction studies. The general features of the molecular structure were reported independently three years ago by Edelman's group at the Rockefeller University (Edelman *et al* , *Proc natn. Acad. Sci. U.S.A* , **69**, 2580; 1972) and by Hardman and Ainsworth (*Biochemistry,* **11**, 4910; 1972) at the Argonne National Laboratory. Recently a series of four papers has come from the Rockefeller group which reports the complete amino-acid sequence of the protein and a definitive description of its molecular structure (Wang *et al., J. biol. Chem* , **250**, 1490; 1975; Cunningham *et al* , *ibid* , **250**, 1503, 1975; Becker *et al., ibid.*, **250**, 1513, 1975, and Reeke *et al., ibid.*, **250**, 1525; 1975).

The X-ray studies have been carried out at 2.0 Å resolution on the tetrameric form of the protein with both types of metal ion bound The overall shape of the monomer is best described as a flat-based dome with dimensions of 42 Å×40 Å×39 Å. Its structure is dominated by two large, entirely antiparallel β sheets. One of these sheets, containing six strands, forms the entire base of the dome and is, unusually, almost lacking the twist normally seen in β sheets. The second β sheet, forming the central slice of the dome, lies at a shallow angle to the first sheet, with its seven strands twisted through 90°. The top of the dome is formed by a number of loops, mainly derived from the immediate N-terminal and C-terminal parts of the polypeptide chain. The quaternary structure of the protein involves two distinct types of subunit interaction, both largely involving the flat β sheet. Interactions of one type cause the monomers to associate in pairs with equivalent strands at the edge of the flat β sheet forming antiparallel hydrogen bonds, to give dimers that contain a 12-stranded β sheet. The second type of interaction involves the face-to-face opposition of these 12-stranded sheets from two dimers, contacts being made through both hydrophobic and ionic side chains that point outward from the sheets. Interestingly the domains of the immunoglobulins, to which the lectins have sometimes been compared, have a similar β-box structure and associate through interactions very like the dimer–dimer interactions in concanavalin A (for references see *Nature,* **253**, 158; 1975). But a more closely related protein is the thyroxine-binding plasma protein, prealbumin, which has both the same type of tertiary structure and subunit interactions (Blake *et al., J. molec. Biol.*, **88**, 1; 1974).

In each subunit the Mn^{2+} and Ca^{2+} ions are located in a surface cavity near the apex of the dome, only 4 6 Å apar
Each ion is in an octahedral enviror
ment composed of four protein ligand:
two of which are shared, and two wate
molecules. The geometry of the bindin
sites seems to explain the ordered binc
ing of the ions in terms of manganes
binding at a proto-binding site, causin
conformational changes that complet
the site and at the same time form th
neighbouring calcium-binding sit
About 20 Å from the metal positions
a deep cavity between the two larg
β sheets in the monomer that seems t
be the saccharide binding site. It seem
to be composed of two distinct subsites
a deeper one that is predominantl
hydrophobic and a more hydrophili
subsite nearer the surface of the mol
cule But although this site is occupie
by the heavy atom labelled inhibitor, *l*
(O-iodophenyl)-D-glucopyranoside, oth
simple sugars that specifically inhib
the protein cause the crystals to dissolv
or lose their diffraction patterns. A
though this behaviour indicates that th
binding behaviour of the protein
altered by crystallisation, Becker *et a*
argue that the alteration is probably
marginal change to the true saccharic
binding site in the cavity, rather tha
the blockage of the true site that
really elsewhere on the molecul
Clearly, this proposal must be tested b
either further cystallographic studies c
by appropriate solution studies such a
NMR, because it is essential to th
understanding of the protein's sug
specificity and its interactions wi
cell surface glycoprotein receptors.

NMR used to study rates of change of conformation

from J. Feeney

A GOOD deal of useful information ca
be obtained from measurements of t
enhanced proton NMR relaxation rat
of the water in aqueous solutions
metalloenzymes containing parama
netic ions In the past these metho
have been used by Cohn, Mildva
Koenig and their coworkers to dete
mine binding constants for the par
magnetic ion–protein complex and t
nature of their ternary complexes wi
ligands. From studies of the frequen
dependence of the relaxation enhanc
ments it is often possible to deduce t
number of water molecules in the c
ordination sphere of the metal, a
detailed information about the liga
exchange rates and correlation times
the interactions. When Barber a
Carver (*J. biol Chem.*, **248,** 335

1973; *Can J. Biochem*, **53**, 371; 1975) applied these techniques to Con A they were surprised to observe large time-dependent changes in the water proton relaxation enhancement after Mn^{2+} was added to a solution of Con A.

Maximum enhancement was noted immediately after the addition and then the enhancement decayed to a reduced value with an apparent half life of ~20 minutes which strongly suggested that a slow conformational change was taking place. They were able to show subsequently that while the occupation of the transition metal binding site does not change the conformation of the demetallised protein, the addition of Ca^{2+} ions to the Con A–Mn^{2+} complex and of α-methyl-D-mannosides to the Con A–Mn^{2+}–Ca^{2+} complex perturbs the protein structure.

Grimaldi and Sykes (*J. biol. Chem.*, **250**, 1618; 1975) have recently developed an elegant stopped-flow NMR method which allows them to follow the kinetics of the various conformational changes induced in Con A by the ordered binding of Mn^{2+}, Ca^{2+} and α-methyl-D-mannoside from measurements of the time-dependence of the water relaxation enhancement under controlled conditions. The sensitivity limitations of the NMR technique often militate against successful kinetic applications but in this case where the intensity of the large water signal is being followed this is not a problem. An advantage of using NMR for such studies is that the NMR parameters for the species involved in the kinetic process contain information about molecular parameters such as number of ligand sites, ligand exchange rates and correlation times for the interaction.

Three separate conformational states of the protein could be discerned in the kinetic experiments and it was possible for Grimaldi and Sykes to outline the order of the steps in the binding process For example, the conformational changes induced by adding Ca^{2+} to Con A in the presence of Mn^{2+} resulted in intensity changes of the water resonance which could be analysed according to first-order kinetics. From the dependence of the observed rate costants on the Ca^{2+} concentration it was deduced that the Ca^{2+} binds rapidly to the Con A–Mn^{2+} complex producing a decrease in relaxation enhancement and this is followed by a subsequent conformation change giving a further decrease in enhancement. The decrease in enhancement probably results from a restricted rate of access of the water molecules to the bound Mn^{2+} following the large conformational change When Ca^{2+} dissociates from the conformationally changed protein the water proton relaxation enhancement remains constant, indicating that the protein retains its conformation.

Grimaldi and Sykes have also examined the kinetics of binding the sugar α-methyl-D-glucoside to Con A in the presence of Mn^{2+} and Ca^{2+}. It should be mentioned that there has been some controversy concerning the sugar binding site in Con A. Measurements of the paramagnetic contribution to relaxation rates of carbon-13 nuclei in α- and β-methyl D-glucopyranosides bound to Con A (Brewer and coworkers, *Biochemistry*, **12**, 9448; 1973) indicate that the sugars are much closer to the manganese ion (mean distance ~10 Å) than was suggested by earlier X-ray diffraction studies (20 Å).

In the kinetic studies of Grimaldi and Sykes the initial binding of sugar was considered to be at a site well removed from the metal binding sites and it does not influence the water signal. There is, however, a subsequent slow conformational change after the sugar binding which increases the water proton relaxation enhancement. This is followed by a further slow conformational change which is essentially the same as that resulting from Ca^{2+} binding in the absence of sugars.

3 . . . 2 . . . 1 . . . 0?

from C H. L. Goodman

ONE-DIMENSIONAL conductors are suddenly the fashionable field of solid state research, and a flood of short but weighty notes in *Physical Review Letters* and *Solid State Communications* warns of massive presentations and theses in the pipeline. It also heralds organic compounds with conductivities like those of metals, and even a completely new type of superconductor.[1]

Electrical conductors normally encountered are relatively isotropic, conducting to a more or less similar extent along any of their three dimensions. Perhaps the only reasonably common material showing pronounced anisotropy is graphite, which in well crystallised material, has an electrical conductivity within the two dimensions of its hexagonal sheets 10^4 greater than perpendicular to them. Such 'layer lattices' have been the subject of a great deal of research over the past ten years. Fairly recently however there has been a sharp switch of attention to 'one-dimensional materials' (composed of parallel long chain molecules).

There are three main materials being studied at present: TTF : TCNQ (tetrathiafulvalene: tetracyanoquinodimethane), the polymer of sulphur nitride (SN)$_x$, and KCP (K$_2$Pt(CN)$_4$ Br$_{0.3}$ 3H$_2$O). All show high electrical conductivity along the chain axis, and rather small conductivity perpendicular to it. They look metallic to the eye, but as might be expected show marked optical anisotropy with polarised light.

Until recently the most dramatic research has been associated with TTF : TCNQ. Heeger and his group at the University of Pennsylvania believed they had discovered a possible organic high temperature superconductor[2] — certainly with a room temperature conductivity of 10^3 (ohm cm)$^{-1}$ rising to nearly 10^5 (ohm cm)$^{-1}$ just below 60 K, this material is a most highly conducting solid and has been much investigated[3]. The sharp transition to a much more resistive semiconductor at 58 K was interpreted as resulting from a Peierls instability — a type of crystallographic distortion which is expected in these materials[4] but which might be avoidable by disordering the material in some way or by modifying the structure. Thus IBM workers have recently reported[5] the transition being reduced to 40 K with the selenium analogue, TSeF : TCNQ.

The platinocyanide, KCP, also shows high electrical conductivity rising sharply as temperature is reduced, but this material (and its chloride analogue) again undergoes a transition to a high resistivity solid. Its study has been bedevilled by its chemical instability: the water molecules which stabilise the structure can get lost well below 100 °C, and in any case the correct structure has only recently been established[6,7].

Probably least understood is (SN)$_x$; it is one-dimensional[8], though with helical rather than linear chains; it is metallic and conducts well even at low temperatures[9]. But what has particularly excited interest is that it has recently been found to be a superconductor[1], albeit with a very low transition temperature (0.26 K), the first quasi one-dimensional superconductor to be discovered. It is interesting that the discoverers refer to the weak inter-chain coupling as a possibly important factor

The key to all these one-dimensional materials in fact almost certainly lies with what are usually termed the van der Waals bonds holding the chains together in the crystals. These bonds are not too well understood and some degree of electron orbital overlap may be involved. It is probable however that crystal growth presents a related but more immediate problem, perhaps a real practical barrier to progress. One cannot but be suspicious that 'one-dimensional' crystals appear to be capable of being 'fattened up' during growth, whereas a truly one-dimensional crystal should not be able to nucleate on its sides and should persist in (linear) growth with the diameter of the seed (assuming that the latter can be obtained). To me the observed 'growing-out' means either that structural imperfections are present due to forced growth or (and it may amount to the same thing) that chain branching can somehow be induced during growth. If this view is correct the most perfect one-dimensional crystals will be those which are most acicular, not the more equiaxed shapes which have been prepared, after much effort, to give large area specimens for physical measurement.

Finally if van der Waals bonds are so important should not someone be looking at zero-dimensional crystals — those held together only by van der Waals forces (or at least what are usually thought of as them)? These crystals by definition would not be metallic, of course, unless 'intercalated' (if that were possible) but their transport properties could be interesting. If a suggestion is in order, GeI_4 and SnI_4 with their brilliant vermilion-red hue do have a most attractive appearance!

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Science and religion

from a Correspondent

For the past three years there have been a number of informal consultations between scientists who have been concerned to relate their scientific knowledge and modes of study to their religious faith and practice. Those involved decided in 1974 that their deliberations should be more widely known and from April 10 to 12, the 'Science and Religion Forum' was inaugurated, at a meeting at van Mildert College, Durham, devoted to 'The Problem of Consciousness'.

THE problem was introduced by a paper on "reductionism in biology" by A. R. Peacocke (University of Cambridge), who distinguished between different forms (ontological, methodological and epistemological-linguistic) of 'reductionism'—roughly, the analysis and explanation of entities in terms of their component units, for example explaining all biology in terms of physics and chemistry. Some forms of reductionism would be generally agreed as simply required methodologically by scientific research, but others represent a more general position which can determine attitudes to the relation between consciousness and brain states. Mary Hesse (University of Cambridge), expanded this discussion by describing certain non-additive, non-reducible situations in the quantum physics of particles. Higher-order relations can emerge in complex (for example living) systems which, not in any mysterious metaphysical sense, constitute a kind of logical irreducibility. For example, the analogy of programming from computer science could be valuable in providing a non-reductive valid way of talking about consciousness.

T. Appleton, of the University of Cambridge, gave an account of studies on communication and language at various levels in the biological world and, in particular, described recent work on primates, which shows evidence of a higher level of consciousness, based on their ability to communicate through American Sign Language, than previously thought from studies of verbal communicating abilities.

Howard Robinson, of Liverpool University, outlined, for the benefit of a predominantly scientific audience, the state of the mind–body problem in contemporary philosophy which has concerned attempts to make materialism philosophically respectable—attempts which though widely canvassed, have made only a limited number of converts and which Mr Robinson rejected. Some form of materialism was, in fact, that adopted by a number of those who joined in a general discussion session which inevitably focused on the sensitive area of concepts such as 'soul', 'immortality' and 'personal identity' in relation to scientific, philosophical and theological ideas.

Professor John Bowker (University of Lancaster) described the development of religious consciousness in terms drawn from systems analysis and information process. He drew attention to the extent to which religions function as bounded systems of information process, in which resources are made available to human beings for the construction of a life-way from birth to death—and traditionally in religions, through death as well. 'Religion' and 'science' were originally undifferentiated, emerging from the general endeavour of men to defend and maintain the continuity of their life-way in relation to the limitations which circumscribe it. The sense of God is effective and dynamic as an informational resource in the construction of human life : to take an example, it would thus be possible to interpret Jesus as the totally God-informed person retrieving in the scan of every situation the cues through which the 'sense of God' is stored in human brain-behaviour, and thus himself becoming a resource for the construction of a religiously-informed human being

At very short notice, the Bishop of Durham, John Habgood, spoke on "religion-and-science and the church". He stressed the need for philosophical sophistication in the science–religion debate; for reconciliation between the idea of continuity assumed by scientists and that of discontinuity implicit in the beliefs of ordinary Christians; and for man's responsibility for nature (including the ethics of research) to be a principal concern of those present.

Two-step transfer reactions between heavy ions

from P. E. Hodgson

LAST year some measurements were made of the differential cross section of the ^{48}Ca(^{16}O, ^{15}C)^{49}Ti reaction at 56 MeV by the Argonne group (Kovar *et al.*, *Phys. Rev. Lett.*, **33**, 1611; 1974). This is a strange reaction: at first glance it looks like a one-nucleon transfer reaction, but closer examination shows that the transferred 'nucleon' must carry two charges. The only possible mechanism is thus a series of nucleon transfer reactions equivalent to a transfer of two protons minus one neutron. This might be expected to be very unlikely, but in fact it is found that the cross section of the reaction to the ground state of ^{49}Ti is comparable to that of normal one-nucleon transfer reactions.

In recent years there has been much interest in multistep contributions to nuclear reactions. These are recognised to be of two types: first those that can proceed through the excitation of the target or the residual nucleus by inelastic scattering (*Nature*, **251**, 188; 1974) and second those proceeding by a series of particle transfer processes. Calculations have been made in a variety of ways for both processes, and in many cases it has been found that the inclusion of multistep contributions greatly improves the agreement between theory and experiment.

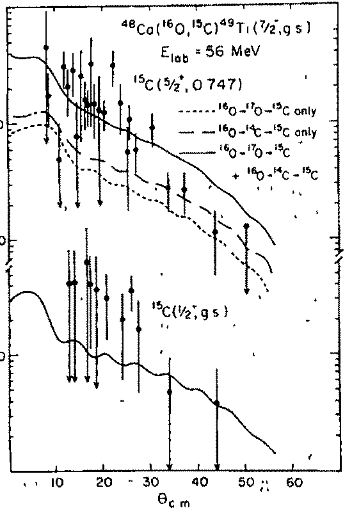
In most of the reactions studied so far both these processes could conceivably contribute, and it is sometimes not clear which is the dominant one, except in reactions involving highly collective nuclei which are very easily excited by inelastic scattering, so that the first process dominates. The special feature of the (^{16}O, ^{15}C) reaction is that it must proceed by successive particle transfer, and so it furnishes an excellent case to test the multiple particle transfer theories.

These calculations are exceptionally complicated because in heavy ion reactions it is not sufficiently accurate to make the zero-range and no-recoil approximations that greatly simplify the calculations for light ion reactions.

The Texas group of Tamura, Udagawa and Low have however found ways of carrying out the full calculation for heavy ions without loss of accuracy and sufficiently economically for them to be done in a practicable time on a fast computer. They have written computer programs to do both types of multistep calculation for heavy ions, and have now applied one of them to the ^{48}Ca(^{16}O, ^{15}C)^{49}Ti reaction (*Phys. Rev. Lett.*, **34**, 30; 1975).

They considered two possible reaction mechanisms, one-neutron pickup followed by two-proton transfer and *vice versa,* and added their amplitudes to obtain the total cross section. Written out in full, the two reaction mechanisms are ^{48}Ca(^{16}O, ^{17}O)^{47}Ca(^{17}O, ^{15}C)^{49}Ti and ^{48}Ca(^{16}O, ^{14}C)^{50}Ti(^{14}C, ^{15}C)^{49}Ti. The intermediate nuclei ^{47}Ca and ^{50}Ti were assumed to be in their ground states and in the first reaction the ^{17}O nucleus could be either in its $5/2^+$ ground state or its first $1/2^+$ excited state, leading respectively to the $1/2^+$ ground state and $5/2^+$ excited state at 0.747 MeV in the final nucleus ^{49}Ti, both of which were observed. In the second reaction the ^{14}C nucleus remained in its ground state.

As the experimental cross sections are structureless and of low accuracy it is not possible to make a good test of the calculations solely by the fit to the angular distribution. It is necessary to try to reproduce the absolute magnitude of the cross section, and this is particularly important because one of the exceptional features of this reaction is the high value of the cross section to the ground state of ^{49}Ti.



Differential cross section for the ^{48}Ca(^{16}O, ^{15}C)^{49}Ti reaction to the ground and first excited states of ^{49}Ti compared with exact coupled reaction channel calculations. The contributions of the two postulated reaction mechanisms are shown separately and added together.

To obtain an absolute cross section all the parameters of the calculation must be fixed beforehand by reference to other reactions. This was done in the present case by fitting available data for the one-neutron transfer reaction ^{48}Ca(^{16}O, ^{17}O)^{47}Ca and the two-proton transfer reaction ^{48}Ca(^{16}O, ^{14}C)^{50}Ti which are the same or similar to those involved in the postulated mechanisms for the ^{48}Ca(^{16}O, ^{15}C)^{49}Ti reaction.

The results of these calculations are shown in the figure, and it is apparent that the absolute magnitudes are correctly given, and that the shapes of the calculated angular distributions are consistent with the experimental results. It is particularly gratifying to find that the relatively high cross section of the reaction to the ground state of ^{49}Ti is correctly given. The relative magnitudes of the two reactions are attributable to complicated interferences between the contributing processes.

More precise data are needed before we can learn more about the details of this reaction, and other possible contributing processes must be considered, but this pioneer calculation of a multistep particle transfer process shows that these reactions are now amenable to quantitative calculations, and it is likely that many more such calculations will be made in the future.

Engine with a solid working fluid

from Robert W. Cahn

THE faintly surrealistic title of this reportage denotes a faintly surrealistic concept. All 'heat engines' hitherto have been designed to operate with working fluids: the fluid may be steam, petrol vapour/air mixture or air (in the Stirling engine), but all depend on cycles involving simultaneous changes of temperature and pressure. Now it is proposed to design an engine in which changes of temperature and stress are applied to a metallic spring, which constitutes the working 'fluid'.

The idea (which is not altogether new) is revived in a paper by Tong and Wayman (*Metallurgical Transactions,* **6A**, 29; 1975). The spring has to be made of one of the alloys—a select band—which exhibit shape-memory. The peculiar properties of these alloys have featured in these pages before (*Nature,* **219**, 895; 1968; **224**, 844; 1969). Essentially, such an alloy can be 'imprinted' with a suitable shape, such as that of a spiral spring, by plastic working followed by heat treatment. It can then be pseudo-plastically deformed at or near ambient temperature: it will retain the new shape until it is heated to a higher temperature (typically 100 °C) and it will then return to its imprinted shape, even against a substantial restraining force seeking to prevent it. It can then be cooled back to ambient temperature without further distortion. Tong and Wayman point out that this sequence can form the basis of a heat engine to utilise solar heat or waste heat. They do not consider the engineering design (though they refer to a properly engineered prototype recently constructed at the Lawrence Radiation Laboratory) but they do attempt to evaluate the ideal cycle efficiency of such an engine as a function of various metallurgical variables, and conclude that an ideal efficiency of about 20% should be quite feasible.

Part of the charm of the idea is that the critical temperatures of the alloy, which define the upper and lower temperature limits of the cycle, can be varied within wide limits, both by going to different alloy systems and by small changes of composition within one system. Nearly equiatomic Ti/Ni alloy (Nitinol) is probably the most promising—and most thoroughly investigated—material; it has a virtually unlimited tolerance of mechanical cycles without fatigue.

The engineering aspects of a solid-state engine will require a good deal of design ingenuity Ideally, it would seem, a simple engine should incorporate two or more springs (or other more elaborate configurations), so that one could be heated up while the other gives up its heat to a sink; heat transfer during both heating and cooling would need to be rapid if the engine is to be anything more than a mechanical tortoise. Presumably a heat pipe to earth would be necessary. An engine of this type might, for instance, be designed to pump irrigation water in deserts, using solar energy.

Shape memory is merely one of four closely linked metallurgical phenomena: thermoelastic martensitic transformations (progressive, thermally reversible shear transformation); pseudoelastic behaviour (large strains, much larger than normal elastic strains, brought about by shear transformations that are themselves reversible with stress); shape memory effect; and a two-way shape memory effect, which involves shape changes induced by temperature changes alone, without intervention of stress. Some alloys show most of these, some show only one; some show an effect in single crystal form, others will do so in the normal, polycrystalline form. The tangled connections between the various effects have been clearly analysed in an important series of papers by Delaey, Krishnan, Tas and Warlimont (*J. mater. Sci.*, **9**, 1521, 1536, 1545; 1974): the authors list all the alloys that have been found to evince any of these effects and analyse the structural, mechanical and thermodynamic features that underly them. These papers are essential reading for anyone who would like to have a go at a solid-state engine. Delaey and his colleagues have provided the steam tables, as it were, of the solid-state engine

Tong and Wayman have recently published another paper (*Acta Met*, **23**, 209; 1975) in which they analyse some of the thermodynamic variables that predispose an alloy to thermoelastic or shape memory behaviour. One interesting conclusion, which is given an interpretation in terms of the 'quasi-chemical' bond-energy approach, is that an ordered alloy (such as Fe_3Pt) has a lower latent heat of transformation, and is more easily able to undergo thermoelastic transformation, than the same alloy in an ordered form. This seems to be a generally valid conclusion.

The solid-state engine may well be able to compete with other physical devices, with or without radiation concentrators, for extracting power from solar radiation. According to Brinkworth (Solar Energy for Man, Compton Press, 1972) such devices have ideal efficiencies in the range ½-1% (for simple thermoelectric generators) to as much as 30% for thermionic generators with concentrators. The latter are likely to be even more expensive on capital than a solid-state engine, and do not directly generate motion. The engine would seem to deserve some further investigation, which will certainly require the collaboration of metallurgists and mechanical engineers.

Successful quake prediction made

from Peter J. Smith

THERE is now ample evidence that some small to moderate earthquakes are immediately preceded by a period during which the seismic velocity ratio (V_P/V_S) in the vicinity is anomalously low. This effect was first observed by Kondratenko and Nersesov (*Tr. Inst. Fiz. Zemli Akad Nauk SSR*, **25**, 130; 1962) in the Garm region of the USSR for events in the magnitude range 4–5; but similar V_P/V_S changes have since been detected in respect of magnitude 2–3.3 earthquakes at Blue Mountain Lake (New York State), the magnitude 6.4 San Fernando earthquake of 1971, the magnitude 5 Bear Valley (California) earthquake of 1972, two shallow earthquakes in Japan and several other events in various parts of the world. The importance of this phenomenon lies in its potential for earthquake prediction, the more especially as the duration of the precursory V_P/V_S anomaly seems to be related to shock magnitude. But can the potential be converted to practice? In each of the examples cited above, the precursory behaviour was detected by data analysis subsequent to the event. Aggarwal *et al* (*J. geophys. Res*, **80**, 718; 1975), however, are now able to report that they have made a genuine and successful prediction.

It was Aggarwal and his colleagues who caried out the original study of the 1971 earthquakes at Blue Mountain Lake, thus becoming the first (*Nature*, **241**, 101; 1973) to confirm the Soviet observations of ten years before. The larger events in the 1971 series were preceded by V_P decreases of 15–20% and V_S decreases of 5–10%. Subsequently, however, the region became relatively quiet seismically until on 14–15 July 1973 the calm was shattered by two magnitude 3.6 and four rather smaller earthquakes. In response to this renewed activity, Aggarwal *et al.* immediately (15–16 July) set up a system of portable seismographs and strong motion accelerographs to supplement (for six weeks) the permanent BML station.

During the first third of this period, the recording system indicated an average S wave–P wave travel time ratio (t_S/t_P) of 1.73 Over the few days following 30 July, however, t_S/t_P decreased to about 1.5. In 1971 the corresponding 'normal' value of t_S/t_P had been about 1.75 and reductions comparable to that now observed had preceded by several days' earthquakes of magnitude 2 5–3.3. Aggarwal and his colleagues thus took the t_S/t_P decrease of 31 July–2 August 1973 to herald a shock of comparable magnitude. On the night of 1 August they therefore predicted that an earthquake of magnitude 2.5–3.0 would occur within a few days.

The maximum magnitude of the apparently impending event was estimated from the inferred spatial extent of the t_S/t_P anomaly In contrast with the situation in 1971, the most distant (common) station in 1973 remained unaffected and thus presumably lay outside the anomalous zone. Assuming the size of the anomalous region to be directly proportional to the magnitude of the responsible event, and comparing the 1973 data with those of 1971, the upper magnitude limit of the expected earthquake was about 3. The minimum magnitude was obtained by estimating the minimum duration of the t_S/t_P anomaly. The 1971 results had showed that, following its initial rapid decrease, t_S/t_P had returned to its normal value rather more slowly and had reached it about one day before the earthquake occurred. As by 1 August 1973 t_S/t_P showed no sign of increasing, precedent suggested that the expected earthquake was unlikely to occur for at least two days, making a total t_S/t_P anomaly time of at least four days. And according to the previously established magnitude–precursor time relationship (Scholz *et al.*, *Science*, **181**, 803; 1973), this would correspond to a magnitude of 2.5.

The earthquake which occurred close to the edge of Blue Mountain Lake on 3 August 1973 had in fact a magnitude of 2.6. Aggarwal and his colleagues are thus the first to predict successfully the place, time and size of an earthquake in the United States and the first to make practical use of precursory seismic velocity changes. Moreover, in their method the precursory interval (whose onset may be detected but whose duration cannot be known until t_S/t_P returns to normal just before the impending event, which may be too late) may be used for predictive purposes before it is complete. If, as seems likely, the size of the anomalous region associated with an impending earthquake is a unique function of the shock magnitude, it follows that once the anomaly appears (and assuming it grows rapidly to its full extent) its spatial dimensions should give magnitude directly. The magnitude can then be fed into the known magnitude–precursor time relationship to determine how long the anomaly will persist and thus just when the earthquake will occur. In short, in any particular case prediction may not have to await the gradual approach of t_S/t_P towards its normal value; valuable time may be gained by making the critical measurements before the rise in t_S/t_P even begins.

Obviously, for this method to succeed it is essential that the size of the anomalous zone be measured reasonably accurately; and here there are difficulties. For one thing, the size of the zone may vary with time, which would complicate the supposed relationship between zone size and magnitude. The temporal characteristics of this relationship are not yet known, although there is evidence that if the anomalous zone grows it must grow relatively rapidly. Second, it would clearly be advantageous to use explosive sources to determine precursory velocity changes and thus define the extent of the anomalous region But in this case care must be taken to ensure that the waves actually penetrate the zone of anomaly. Aggarwal *et al.* show that large changes in V_P and V_S are not observed at shallow depths (less than 0.5 km at BML) for near-horizontal wave paths. This could mean either that significant velocity anomalies do not exist at these depths or that the cracks there are predominantly horizontal. Either way, some sort of anisotropy is implied; and this is likely to complicate the mechanics of the prediction method.

Manpower supplement

In this issue we put together a number of articles on different facets of scientific manpower. This can in no way be regarded as a comprehensive survey; rather we have considered a number of subjects and of countries and have taken one person's view in each case. The treatment is very largely journalistic—if that means that our analyses lack the background of years of experience it also means that they lack much self-congratulation and a sense that all is necessarily for the best. For in some ways the purpose of this supplement is to warn that the question of the use of scientific manpower is increasingly not one which can be ignored. It is becoming abundantly clear that the system by which schools supply universities and polytechnics which supply schools, industry, government and themselves is not automatically self-regulating.

At the heart of manpower issues are several contradictions:

- Scientists or their representatives are increasingly looking for life-long tenure, although science itself proceeds more by revolutions and perpetual rejuvenation.
- The waning appeal of science to the young is widely believed to be largely because science is highly specialised and dissociated from human dimensions and therefore unattractive, but this is just at a time when some of the questions that are being asked of science are simple, stimulating, strongly related to human needs, and highly multidisciplinary.
- Bad news about employment prospects can affect the choice of career of a prospective scientist six or more years before employment prospects become relevant.
- At a time when the techniques of manpower planning are being criticised for coming up with the wrong answers, the British Department of Education and Science apparently intends to use the manpower-planning approach in formulating some of its policies for higher education.
- Academic life is supported in great part by the taxpayer and yet intervention by government in university affairs is all but unthinkable.
- Scientists, particularly in government and industry, often experience a conflict of loyalties between their employer and the wider, often international, cause of science.
- Students, for various reasons, can occasionally get hooked in large numbers on subjects such as astronomy in which employment prospects are poor and universities often powerless to do much except absorb all-comers.

Perhaps one of the most tricky problems of manpower use is the all-growing-old-together syndrome. This can be seen around the world in two totally different situations—defence establishments and new universities. In the 1940s, in response to the demonstrable success of scientists in the Second World War and the emergence of the Cold War, many new establishments were formed and many old ones recruited in large numbers. Likewise in the 1960s in Britain (and later in some other parts of the world) many new universities were built and absorbed vast numbers of young researchers as teaching staff. The degree of homogeneity in both these groups is undoubtedly higher than elsewhere, but homogeneity coupled with a growing seniority is not necessarily the best of environments for research. And recruitment within a narrow temporal band often means that people are taken on simply because they are around rather than because of any intrinsic merit they possess.

There are many other topics worth pursuing—brain drains, the PhD in industry, retraining programmes, the research scientist as school teacher, are but a few. The only things that can be said with much certainty about the scientific profession at present are that a young man entering at 21 can not expect an uninterrupted ride to 60, and that the scientific community is going to hear more and not less of the word manpower in the next few years.

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Excess demand in the graduate employment cycle

John Hall

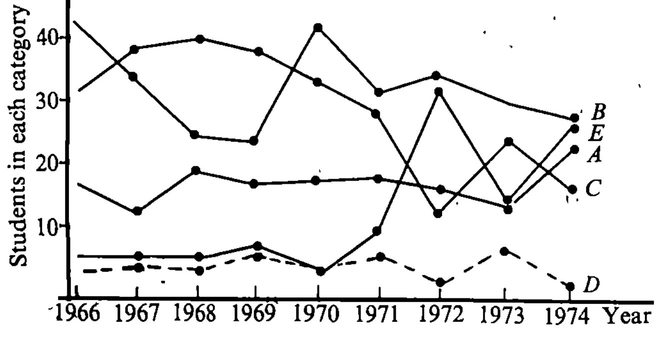
After the lean years of the early 1970s the traditional employment of science graduates is picking up, but the result is likely to be a shortage because the graduates have found that non-traditional jobs can be quite attractive.

THREE years ago, anyone embarking on an account of how scientific manpower problems were affecting British university science departments would have had a field day. The big twelve companies who normally take most of the country's new science graduates had developed cold feet because of an acute economic recession, and the last people they needed on their payrolls were recently qualified specialists who knew next to nothing about industry and would take at least two years to start paying their way. Some companies pared their intake dramatically, others simply said they would take no chemists, or chemical engineers or metallurgists at any price. Alarming noises about the growing pool of unwanted science graduates were to be heard from universities like Manchester, and nobody dissented too vigorously from the view that there would never again be a return to demand of the mid-1960s for chemistry graduates.

Up and down the country, careers masters read their newspapers and sixth formers listened to hard-luck stories from 'old boys' who were selling vacuum cleaners door-to-door because there were no more laboratory jobs available and, shortly, entrants were avoiding the worst-affected courses in such numbers that chemistry professors began to look nervously over their shoulders in case anyone noticed that there were not all that many people to lecture to any more. (The staff–student ratio was a nonsense in an area which had an important research function, they implied, and in any case, as one professor pointed out in a letter to *The Times*, the laboratory assistants on the payroll made the picture look bleaker than it really was.)

Now it is the turn of the science-based industries to sweat things out, and university chemistry and chemical engineering departments are in the position of supplying an increasingly scarce product in a seller's market. The better graduates are being offered a choice of up to six jobs this year, and in 1974 chemical engineering graduates at the University of Manchester Institute of Science and Technology (UMIST) were accepting first appointments at around £3,000 a year. One started at £5,000. The only people who seem surprised by the turnaround are the unfortunate recruiting officers who closed industry's doors so recently and announced that the golden days were over. Now they have the doors open again, and they are explaining that they had never meant that they should be taken so literally when they said what they did. But nobody (in the universities at least) is shedding too many tears over industry's plight, because there is a fairly unanimous opinion that the wound was self-inflicted. What is more, many departmental heads and appointments board people went to great pains in 1971 and 1972 to point out to bashful employers the likely effects of over-reacting, and now those effects are to be observed, precisely as predicted, they can be excused a little self-congratulation.

Fig. 1 First destinations of graduates of the University of Manchester. *A*, Teacher training; *B*, postgraduate work; *C*, chemical or allied work; *D*, 'still looking' six months after graduation; *E*, commercial or other work.



In hard times, the universities said, industry would clearly be making some cuts in its recruitment programmes. If those cuts were reasoned and moderate, there would be a corresponding feedback to the schools, where there would be a gradual and appropriate decrease in the number setting out to be chemists (or whatever). According to this picture, industry's decreasing demand and the universities' decreasing supply of scientists would describe similar trajectories on a graph of the affair, levelling off to run in parallel towards an infinitely distant point of mutual satisfaction.

An alternative approach was for industry to base its recruitment plan on that year's trading figures, without reference to much else. In this situation you cry out for all the manpower you can lay hands on when business is going well, and when the books begin to look bleak you reduce recruitment drastically, or if it is a really bad year you cut it out, period. Now, the news gets back to the schools that there is simply no way in which anybody is going to make a living as (say) an industrial chemist. The vacuum cleaner salesman confirms what the careers master has read and, instead of getting a tapering off of supply, the universities find that the bottom drops out of the market in the appropriate subject. This would be an admirable approach if it took twelve months to train a chemist from his entry into the lower sixth form at school. As it happens, it takes five years for subject options made at school to be translated into terms of graduate supply, so if industry were to take this line then it would be fairly crucial that it be absolutely certain of its manpower requirements five years ahead.

In the event, science-based industries proved to be no better than anyone else at forecasting needs five years in advance, and as a result it is now in the market for people whose scarcity is a direct result of its own short-term manpower policies. Because of the time lag between the industry's statement of its manpower requirements (or non-requirements) and the effect of that declaration on graduate output, the graph shows two curves in constant oscillation about an axis which they both cross about once in five years. Industry pays through the nose when the scientists' supply curve is at its nadir, and the demand curve peaks. When it happens the other way round, the scientists pay for it.

Responses

As university students and teachers are thinking beings, and not the mute commodities which the supply and demand equation supposes, however, they are able to make adaptations to reduce their vulnerability, and since they received their last fright from industry about four years ago there have been at least two changes which strengthen their position. Industry's recruitment cut-back made many science-trained graduates look for jobs in areas which were, traditionally, beyond both their area of interest and their grasp. They moved, often out of sheer desperation, to the professions which offered a kind of institutionalised security and which had been historically the preserve of the arts graduate—that is, to the Inland Revenue, to insurance, business management, accountancy, and even law. These new directions, although often taken as makeshifts, have been found by science graduates to be surprisingly pleasant alternatives to the accepted routes through the research and development or academic career structures—so much so that although the necessity for science graduates to seek non-traditional alternatives as their first-destination employment has now been largely removed, there has not been a corresponding switch back to the traditional jobs. In effect, it has been established that a scientific education may be regarded as a non-vocational training for a variety of non-scientific careers.

Table 1 Possible employment of new chemical engineering graduates in 1978

Public service and education			25
Public Service			
Civil Service, local authority, river boards, and H.M. forces	20		
Education			
Technical college, *etc.* (not teacher training)	5		
Industrial and commercial			440
Oil and chemical industry		180	
Heavy chemicals	72		
Fine chemicals	30		
Petroleum	40		
Plastics	15		
Industrial gases	15		
Soap and detergents	8		
Engineering		120	
Contracting	60		
Chemical plant manufacture	25		
Steel and other metals	28		
Air conditioning	7		
Other manufacturing		60	
Food	20		
Fibres and textiles	15		
Paper and board	10		
Others	15		
Civil Engineering, building contractors, and so on		5	
Public utility, NCB, AEA		40	
Coal	12		
Other mining products	12		
Water and effluent	12		
Atomic energy	4		
Accountancy		15	
Banking and insurance		5	
Other commerce		15	
Others			5
Total entering permanent employment in this country			470
Employed overseas (on permanent or short term basis)			40
Returning home overseas or otherwise not available for employment			40
Failing to keep in touch or taking more than 6 months to find permanent employment (as in recent surveys)			100
Higher education and training (including teacher training)			180
Total output of graduates required			830
This total is based on a distribution of home to overseas students of 730 home to 100 overseas			
1975 intake to university chemical engineering departments required to provide this output		950	

The Institution of Chemical Engineers, which is keen to see a restoration of equilibrium in the demand and supply of new graduates, conducted an investigation leading to an estimate of the demand for graduate chemical engineers in 1978 (published in *Chemical Engineer*, March, 1975). A report presented to the institution's Careers Committee was prepared, by way of a preliminary estimate, by combining first-employment surveys and assessments by industry and Institution members. The resulting Table above is believed to be a conservative estimate of the possible destination of new graduates and shows that the universities should be producing about 830 new graduates each year. Allowing for the usual wastage rate, the intake to universities should be in the order of 950. The actual intake could be as much as 400 short of this target.

This response to a scientific manpower surplus was foreseen as long ago as 1961, when a government report (*The Long Term Demand for Scientific Manpower*) welcomed the possibility of scientists moving into non-scientific careers. A surplus of scientists over immediate demand for employment, said the report, "should make possible a rational, as opposed to an emergency use of scientific disciplines. It should mean that at long last we shall have a supply of qualified manpower with a scientific training for management, administration and the professions generally, in addition to those who up to the present have been drawn inevitably into vocational employment. We do not doubt that scientific education will adjust itself to this new prospect; and that in the same way as only a proportion of those trained in the classics and history have expected to find employment in their own fields of study, an increasing proportion of those trained in specialised scientific disciplines will obtain employment outside them. We think that both science and the nation will benefit from this adjustment."

What the student employment applications for 1974 and this year show is that the trend foreseen in the 1961 report as a response to a manpower surplus situation is continuing at a time when the conditions which gave rise to it have subsided. A second change which has occurred as a result of the falling demand for scientists at the beginning of the decade is that course content has changed (and in some cases entry requirements) in order to secure students with a broader initial outlook and a broader and more flexible operational capability on graduation. The notion that it is possible to predict when a student enters, the market forces which will prevail when he leaves university has in many places been abandoned; the aim is rather to give the student a hard core of indispensible knowledge in his special area, and to offer a range of options which will make him sufficiently versatile to take advantage of such employment opportunities as present themselves, whatever they may be.

Trends

Apart from making the science graduate a more adaptable individual than he was formerly, the cut-back in demand has also tended to make him think in a very positive way about the direction he wishes his career to take. Whereas during the 1960s anyone going to university to read chemistry accepted without much thought about the matter that he would work as a chemist in industry, in government research or in a university, that assumption is no longer made today. Appointments board officers at Cambridge, Manchester and Imperial College, London agree that the changed circumstances of the science graduate in the early 1970s have produced a situation in which the student has become accustomed to making an active decision about the way his working life will start. They also agree that, although this may be bad news for ICI, or Shell, or for the government centres which automatically expected a visit from the best students every year, it is good news in an educational (or existentialist) sense that people are exercising a more conscious choice about their personal destinies.

On the same level, there is also a feeling that student intake to science has not taken such an upward turn as employment vacancies because of a positive decision among school leavers that science is directly responsible for many of society's ills, and that they will not be a party to any extension of the technological nightmares which beset the Western world. The universities tend to point out, without any sense of historical conviction, that scientists are the only people who are ever likely to make the bad dreams go away, which is rather like saying that peace can only be enforced by the military. And they try to court the science-shy with courses on environmental studies, pollution control and energy sources—which is progress, of a kind.

What universities think

Specific information about science students, courses and employment trends at Manchester, Cambridge and Imperial College can be summed up as follows:

- **Cambridge.** Science courses are generalised until the third year, when a specialist option is taken. The number taking chemistry is so small that professors are starting to worry about the security of their salaries and employers are falling over themselves to get at the few who emerge with chemistry degrees. In the past, the pressure has been on people who are doing fairly well at their subjects to pursue them to the bitter end; that is, to take their higher degrees and go into academic work. But this year it is noticeable that the academic world is not proving as attractive, which is hardly surprising when final-year students looking for openings in university teaching see nothing more rosy than appointments on a one-year tenure. (Professor A. B. Pippard is reported to have said that there is no future in the universities for 90% of the science students who are there now, and that looks like an optimistic estimate.) The consequences of the last cut-back are to be seen in student career choices (accountancy, business and so on) and in the attitude of employers from these areas, who are prepared to regard science as a general training in thinking and, therefore, as an acceptable entry qualification for a variety of non-scientific jobs. Buoyant areas are electronics and communications. General prognosis for the science subjects: an upturn in intake and in employment opportunities, but never a return to the levels of the 1960s.
- **Imperial College, London,** prides itself on having a finger on the pulse of industry, and for this reason claims that it never had any real problem placing its graduates although there was a distinct drop in the number of applicants for chemistry courses last year. Maybe it never had much trouble in its placings because it has a tradition of sending people to a wide range of careers (up to 20% make for areas like banking and insurance). They report that since people tried teaching as a life-raft during the lean years it has become a popular career choice on its own merits. The demand for chemists and chemical engineers is well up, and Imperial College says it did not really notice a serious decline in the demand for chemists at any stage. (In the 1970–72 period when the national unemployment figure stood at 16%, Imperial College's graduate unemployment was less than 0.5%.) In its best years, the chemistry department had up to 1,000 applicants for 95 places. This fell in 1974 to 220-odd applicants for 60 places, but this year applications are up 30% on last year. Imperial College sells its graduates by emphasising project work, individual initiative in problem solving, and practical laboratory experience. It also makes a good showing on integrated studies like the history and philosophy of science and languages.
- **Manchester.** The university hopes the decline in chemistry applications has bottomed out and is on the increase again. This year there are 60 final-year chemists, compared with 80 to 95 in the best years. There is a demand for Manchester's chemists, who now do a course of two years' hard-core chemistry, followed by a year of options which include environmental chemistry, psychology, management and liberal studies. Flexibility is the keynote. The metallurgy department, with less than half the number of students it had in 1972 (and with the national total of metallurgists lost running at 250 a year), is in a position to take its pick of the jobs offered. The subject's unpopularity is partly caused (according to Professor K. M. Entwistle) by "a reluctance to inject courses with an element which lets the students see that we recognise the responsibilities of science; that we see science as more than an intellectual exercise." Remedy: broader-based courses including economics, accounting and social science. This year's applications are up by more than 50% to something over 200. Image is also a problem in the chemical engineering department at UMIST, where it is feared that anything with the word 'chemistry' in it is equated with the word 'poison'. A broader course is seen as part of the solution again; this time it is subjects like pollution control, energy sources and foreign languages as a relief from the narrower mainstream of chemical engineering. A second brain drain is forecast when the Americans come to Britain to recruit for their energy-crisis programme, and since there is already a shortfall in chemical engineers produced, as against numbers required, they will become an even scarcer commodity than they are this year, when they can take their pick of jobs. Five years ago they were next to unemployable. Every other applicant for chemical engineering in the UK applies to UMIST, and what he gets for his money is an unsurpassed pilot plant to practice on, and a course with an entirely elective third year.

Signs of shifts in employment patterns

Colin Norman

After a period of rapid growth, enrolment in higher education in the United States is levelling off. The result is likely to be an increasingly bleak academic job market for scientists and engineers with PhD degrees and the trend has major implications for the nature of graduate education.

LAST month, a committee of eminent astronomers issued an unprecedented appeal for graduate education in astronomy to be cut back because of dismal employment prospects for new PhDs. Unless enrolment in graduate astronomy departments is substantially reduced, the committee said, the number of young scientists leaving universities in the United States with freshly minted PhDs in astronomy and closely related subjects could soon outstrip the number of available jobs by about six to one. Already, the committee noted, there are some 200 astronomers holding temporary postdoctoral appointments who have little chance of getting permanent jobs in astronomy.

Although extreme, the manpower situation in astronomy reflects problems which are being encountered in some other fields of science. In fact, if most projections are at all accurate, there is likely to be a growing imbalance between the supply of scientists and engineers with PhD degrees in a variety of disciplines and the number of jobs for which their training is directly suitable.

That prediction is based on two major trends affecting the manpower situation for PhD scientists and engineers. First, federal expenditures in most areas of science and technology have recently levelled off or even declined, and there is unlikely to be much real growth in support of basic research in the next few years. And, second, after a period of rapid expansion, higher education has entered a period of retrenchment. Overall enrolments in graduate and undergraduate departments have been declining and there is little chance that they will grow much over the next 20 years.

Although the reduced enrolment will tend to curb growth in the supply of scientists and engineers, it will also have a marked impact on the academic job market for PhDs—the declining growth rate in higher education will result in a similar reduction in new teaching positions in the universities, so that scientists and engineers emerging from graduate schools will increasingly have to look outside academia for employment. Superimposed on that trend, however, is the fact that prospective federal support for a few activities—such as energy research and development and possibly cancer research—is likely to create high levels of demand for scientists and engineers with both first and higher degrees in a few selected disciplines. In fact, according to some estimates, there may be severe shortages of people with some specific skills and that could distort the overall manpower picture drastically.

Those trends hold interesting implications for higher education and it should also be noted that they are both outside the direct control of the universities. The slowdown in overall growth is largely a consequence of the fact that the postwar 'baby boom' has now passed through higher education so that there is likely to be no growth in the college-age population for at least the next two or three decades. And the increasing support for energy research and development is coming directly from the federal government.

Boom and bust in the 1960s

In view of distortions created by previous federal programmes, college administrators are likely, however, to view with mixed feelings the prospective rapid increase in support for energy research. The situation which led to the call for cutbacks in enrolments in graduate astronomy courses is instructive in that regard. The call came from a special committee of the National Academy of Sciences, which met under the chairmanship of Leo Goldberg, Director of the Kitt Peak Observatory.

Like most other sciences, support for astronomy expanded rapidly during the early 1960s as part of the frantic post-Sputnik build-up of science and technology in the United States. But, just as young people began pouring out of the universities with PhDs in astronomy in the early 1970s, support for astronomy began to decline. The figures speak for themselves.

The number of PhDs employed in astronomy increased by 111% (from 623 to 1,313) between 1970 and 1973, and last year alone 180 astronomy PhDs were awarded. There are now about 900 students enrolled in graduate astronomy courses who will be looking for jobs in the next five or six years, and adding to the problem is the fact that astronomy has traditionally attracted PhDs from closely related branches of physics. Yet the committee estimates that there will be no more than 200 jobs available in the next five years.

At the root of the problem is the fact that funding for astronomy in general has been declining—federal support shrank from $227.3 million in 1968 to $186.8 million in 1972, the committee estimates. And there are scant prospects of any increase in the years ahead since the bulk of the funding now comes from NASA; with the shuttle taking up an ever-increasing share of the space budget, there is likely to be little money to spare for expanding space-borne astronomy, at least until the early 1980s.

Although it can be argued that astronomy is something of a special case, since job openings are largely confined to one sector—higher education itself—the situation there dramatically underscores one basic factor in supply and demand for highly trained manpower. The education process is so lengthy that periods of high demand and plentiful supply of trained individuals are often completely out of phase. Moreover, the trends which have led to the bleak manpower situation in astronomy—rapid expansion, followed by cutbacks, followed perhaps by constant or at best slowly increasing support—are evident in most scientific disciplines.

Overall, US expenditures on science and technology expanded rapidly during the early 1960s, but began to level off in about 1968. In fact, in terms of constant dollars, the total support for research and development declined between 1968 and 1971 before picking up slightly. The same pattern is evident in the total support for all higher education—the share of the gross national product devoted to it doubled between 1961 and 1972, from 1.1% to 2.2%, but has declined slightly since; and total enrolment in colleges and universities, after more than doubling during the 1960s, is levelling off.

In short, just as the universities were reaching peak output of graduates with first and advanced degrees in science, federal support began to shrink. The chief driving force behind those trends was the spectacular expansion of the space programme and its equally spectacular decline in the late 1960s. The result, of course, was that a huge oversupply of scientists and engineers began to emerge in 1970 and 1971 and much publicity was given to unemployment of highly skilled manpower.

According to surveys conducted by the National Science Foundation (NSF), in the spring of 1971 2.6% of all scientists and engineers with first or higher degrees were unemployed and looking for work, and in June and July of that year 3.0% of all similarly trained engineers were out of work. Younger scientists and engineers were the most severely affected (unemployment rates of 5.3 and 5.5% respectively) while PhDs only experienced unemployment rates of 1.4 and 1.9%. Although unemployment among scientists and engineers was running well below the national average (in excess of 6% at that time) the plight of unemployed scientific workers received widespread attention in the press and that may have contributed to declining enrolments in the early 1970s.

Two years ago, the National Science Board, the top policy-making body for the NSF, summed up the situation thus: "Substantial changes in the demand for scientists and engineers, which may be produced by factors such as a redirection in federal funding or the state of the national economy, may occur over periods of from two to three years. But because scientists and engineers require training extending over several years, serious imbalances of supply and demand, inadequacies of training, maldistributions among areas of competence, and similar problems may be corrected only over a longer five- to ten-year span."

A number of warning signs concerning the slow-down in the growth of higher education did, however, begin to turn up unexpectedly in the late 1960s, before the unemployment situation arose. According to data discussed at a meeting last year of science manpower analysts by Alan Cartter, of the University of California, Los Angeles, the following shifts took place.

The proportion of 17- and 18-year-olds graduating from high schools peaked in 1968 and has declined since; analysts had

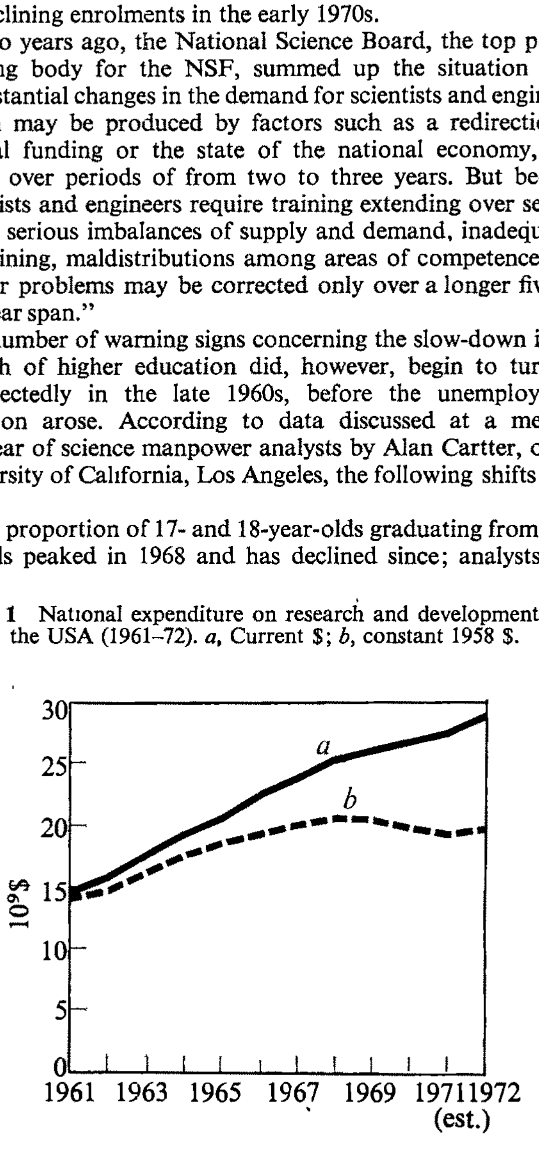


Fig. 1 National expenditure on research and development in the USA (1961–72). *a*, Current $; *b*, constant 1958 $.

Table 1 Summary of science/engineering doctorate labour force and use (1972 and 1985)

	Total	Physical sciences	Engineering	Mathematics	Life sciences	Social sciences
			1972 Estimate (thousands)			
Labour force	221	65	34	13	57	53
No. used as scientists or engineers	206	61	32	12	54	47
No. not used as scientists or engineers*	15	5	2	1	3	6
No. used as scientists or engineers as percentage of labour force	93	93	95	96	95	89
			1985 Probable model (thousands)			
Labour force	375	85	63	22	92	113
No. used as scientists or engineers	293	76	45	16	85	71
No. not used as scientists or engineers*	82	9	18	6	7	42
No. used as scientists or engineers as percentage of labour force	78	89	71	73	92	63

*Includes unemployed.

predicted a yearly increase of 1% until 1980. That was followed a year later by a sudden downturn in the proportion of high school graduates entering colleges and universities—again, the proportion had been expected to increase but between 1970 and 1973 it decreased from 61.8% of all high school graduates to 57.6%. That downturn was itself followed in 1970–71 by a peak in the college graduation rate. And, as for graduate education, enrolment in graduate courses peaked in 1967–68 and has generally been dropping ever since. Moreover, the PhD completion rate peaked in 1969–70.

Those trends have not been consistent among all the sciences. Enrolments in the life sciences and social sciences have generally held up, for example, and the number of women entering and completing graduate courses has continued to increase.

The latest survey of enrolment in graduate education, conducted by the NSF, indicates, for example, that enrolment in graduate engineering courses declined slightly last autumn and enrolment in physical sciences held about steady. But the number of students entering graduate social science departments rose by about 4.4% and enrolment in graduate biological science courses shot up by 17.2%.

Nevertheless, surveying future prospects, Cartter concluded that there is likely to be a slight contraction in total enrolment in higher education at least until the mid 1990s, which will depress the academic labour market. The oversupply of doctorates in the general academic labour market looks grimmer for the period 1980 to the early 1990s than it has at any time in the past.

That prediction is supported by at least three authoritative surveys. Last year, the Office of Education came up with projections of college enrolment in 1980 which suggest that the net increase in the number of full-time faculty members in the entire decade from 1972 to 1982 will be 34,000. That is fewer than were hired in the single year 1965 and compares with an addition of 211,000 full-time faculty members between 1962 and 1972.

More recently, the Carnegie Commission for the Advancement of Teaching predicted that enrolments in higher education are likely to grow slowly, if at all, during the next 10 years, after which they will probably decline. Slow growth may, however, be resumed after about 1995.

Finally, the NSF last month issued some grim predictions concerning the manpower situation for PhD scientists and engineers in the next 15 years. Those forecasts (*Projections of Science and Engineering Doctorate Supply and Utilisation 1980 and 1985*, NSF 75-301) indicate that there is likely to be a growing imbalance between the supply of PhD scientists and engineers and the number of job openings for which their skills will be required.

Specifically, the NSF anticipates the following developments:

- Based on an analysis of trends during the past five years, there are expected to be between 375,000 and 400,000 scientists and engineers with PhD degrees available to the economy by 1985. But there are likely to be only about 295,000 available positions in activities related to science and technology.
- That projection suggests "a trend toward increasing imbalances between supply and utilisation, which would result in more non-science and engineering utilisation of science and engineering doctorates, possibly in some unemployment".
- The NSF report notes, however, that the magnitude of unemployment is difficult to predict but it is likely to be relatively small since people with PhD training are likely to find some kind of employment, albeit outside science and technology or in occupations for which they are overqualified. In that regard, it should be noted that general levels of unemployment among scientists and engineers is now low, in spite of the fact that the United States is in the middle of a prolonged recession.
- There is likely to be a substantial shift in employment patterns, with increasing numbers of PhD scientists and engineers finding jobs outside higher education, or in jobs unrelated to science and technology. In fact, more than half of all new job openings for science and engineering PhDs are likely to be outside research and development in the next decade so that by 1985, more than a fifth of the entire doctorate science and engineering labour force will probably be employed in occupations unrelated to science and technology. Less than 10% of all PhD scientists and engineers are now so employed.

Those projections immediately raise a fundamental question about the nature of graduate education, namely, is the system which has traditionally produced people almost exclusively for academic and industrial research and development also suitable for producing PhDs for other occupations? Almost everybody who has studied the situation believes that a number of changes are required.

In addition to recommending cutbacks in enrolment, for example, the academy's astronomy committee also urged that PhD astronomy courses should be broadened so that the three-quarters of all would-be astronomers who will fail to get jobs in astronomy will be more employable elsewhere. Moreover, an *ad hoc* committee of the National Science Board recently recommended that "educational alternatives be provided to the traditional highly specialized PhD program to prepare students for a broad range of careers".

Those overall projections mask the fact, however, that there are likely to be shortages in a few fields because of rapidly increasing federal expenditures on a number of key programmes. Just as the space programme distorted the scientific manpower situation during the 1960s, the energy research and development programme and perhaps the cancer programme are likely to distort manpower demands in the next decade.

Although it is difficult to predict exactly how many trained people will be needed to carry out those two programmes, two recent studies have raised warning signals. As far as energy research and development is concerned, the National Planning

Association (NPA)—one of the better diviners of manpower trends—has concluded that "the future supply of engineers and scientists may be inadequate to meet the demands of activities designed to increase domestic energy supplies".

In short, the NPA reckons that the energy industry will need to recruit about 14,600 engineers and about 5,500 scientists a year by 1985. But, if recent enrolment trends in undergraduate engineering departments are maintained, the study suggests that only about 28,000 engineers will graduate from American universities in 1985. In other words, the energy industry will require more than half of all the engineering graduates, and about a third of all the graduates with degrees in the physical sciences. In 1970, by comparison, the energy industry employed only 10% of all engineers in the United States and 15% of all physical scientists. During the early 1960s, when there was high demand for scientists and engineers, many companies in the United States recruited from abroad; but the NPA study notes that supply of foreign engineers and scientists is diminishing.

There are, however, some indications that enrolment in engineering courses is beginning to pick up, perhaps in response to the possibility of a favourable job market. The Engineers Joint Council, for example, estimates that the freshman engineering class this year is 18% larger than last year's, and if that trend continues, the NPA's dire prediction that total output of engineering graduates will drop to 28,000 a year may turn out to be wide of the mark. The Engineers Joint Council also noted, however, that the declines in enrolment during the late 1960s and early 1970s resulted in a 5.5% drop in engineers graduating last year. Overall, 41,407 bachelors' degrees were awarded in engineering last year, 15,885 masters' degrees and 3,362 PhDs.

As for the cancer programme, the National Cancer Program Plan suggests that about 13,500 biomedical scientists with PhD or MD degrees will be required for cancer research in 1982. There were only about 5,500 scientists engaged in cancer research in 1972, and the plan suggests that "a deficiency in the number of scientists may begin to occur in [fiscal year] 1975 and may continue to increase as the program expands". It should be noted, however, that funding for cancer research is unlikely to grow as rapidly as the plan indicates and so manpower requirements will also grow rather more slowly. Moreover, enrolment in both undergraduate and graduate biology departments is continuing to increase. The NSF's projections for PhD scientists therefore indicate that there will be a close match between supply and demand for biological scientists in 1985.

In view of those predictions, it may well be asked what the federal government should do to bring supply and demand more closely into balance. First, it should be pointed out that the government's leverage on the supply side is surprisingly small. During the 1960s, for example, in spite of massive government support for science and technology, which included direct support of postgraduate students, the proportion of science and engineering graduates, as a percentage of the total numbers of graduates, remained constant (about 30% of all bachelors' degrees were awarded in science and engineering) whereas the proportion of PhD degrees in science and engineering even declined after 1962. Aside from direct support of postgraduates in energy-related fields, the federal government has recently phased out most of its direct support programmes for science and engineering students (much against the wishes of the universities, which have lost a useful source of income).

The demand side is likely to be influenced chiefly by enrolment patterns in higher education and by total government support for science and technology. As noted above, however, there is likely to be little growth in the college-age population and, therefore, in higher education. As for total government support of science and technology, if recent trends are anything to go by, a period of slow growth is likely to be in store for most areas of science, which at least will be an improvement on the stop-start trends of the late 1960s and early 1970s.

Indian scientific and technical personnel abroad

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Although India educates large numbers of scientists and technologists, there is a considerable brain drain as a result of lack of opportunities for employment. To make full use of their trained manpower, the Indian government has started some schemes to help scientists who wish to return home.

MANPOWER being an essential input for any developmental programme, India adopted in the post-independence years a programme of rapid expansion of education in science and technology to meet the requirements for development of science and technology. The number of universities was gradually increased. Capacities for postgraduate education in the basic sciences were extended and the scope of technical education was broadened through the updating and expansion of the existing institutions and establishment of new ones A number of Regional Engineering Colleges and a chain of five Indian Institutes of Technology (IIT) came into being and the output grew rapidly each year. Table 1 illustrates the growth of universities and other institutions of higher learning and Table 2 the output, at different academic levels, of scientists, engineers and medical personnel. Although there were only 20 universities in India in 1947 the number had risen to 86 by 1972. Higher education and research in professional subjects such as agriculture and engineering attracted particular attention and some newly established universities were exclusively

Table 1 Growth of educational institutions in India

	No. of universities, IITs and other institutions deemed as universities			No. of institutions offering postgraduate courses in engineering	No. of medical colleges
	Universities	IITs	Other		
1945	17	—	—	5	15
1947	20	—	—	5	22
1950	27	—	—	6	29
1952	30	1	—	10	30
1954	31	1	—	14	35
1956	35	1	—	21	46
1958	40	2	2	26	51
1960	45	4	2	30	62
1962	55	5	4	33	71
1964	62	5	8	43	81
1966	68	5	8	56	90
1968	76	5	9	62	92
1970	83	5	9	67	95
1972	86	5	9	77	99
1974	86	5	9	80	105

Table 2 Output of scientific and technical personnel

	BSc	BSc Ag	MSc	MSc Ag	PhD Science	PhD Ag	MBBS	MD/MS	BE/BSc Eng. & Tech	ME/ MTech Eng./Tech	PhD Eng.
1945	4,406	403	745	39	NA	NA	609	NA	987	23	NA
1947	5,996	535	905	79	NA	NA	959	NA	1,076	30	NA
1950	9,628	1,000	1,425	154	NA	NA	1,557	88	2,065	100	NA
1952	11,087	870	2,129	223	108	5	2,164	113	2,882	118	12
1954	14,427	910	2,911	208	164	4	2,582	110	3,104	147	19
1956	16,126	886	3,255	214	210	11	2,732	171	4,163	278	23
1958	18,920	994	3,841	313	216	8	2,839	281	4,237	378	20
1960	22,693	1,700	5,382	488	361	11	3,387	397	5,660	606	18
1962	26,930	2,612	7,218	704	435	50	3,945	525	8,216	756	20
1964	34,046	4,099	8,882	823	537	19	4,452	771	9,387	665	26
1966	42,437	5,259	10,008	1,191	774	94	6,558	1,049	13,015	980	39
1968	59,606	6,239	12,682	1,372	1,100	120	8,916	1,254	15,771	1,076*	66
1970	83,610	5,909	16,578	1,670	1,212	217	9,562	1,266	17,748	1,231*	98
1972	NA	NA	NA	1,945	1,310	309	10,315	NA	16,487	1,408*	76
1974	NA	NA	NA	2,267	NA	NA	11,024	NA	14,704	1,610*	NA

*Estimated. NA, not available.

for this purpose. The IITs were established as centres of excellence for education and research in engineering and technology. In the field of medical education, the number of colleges offering degree-level education rose from 15 in 1947 to 105 in 1974 and a few institutions were established exclusively for postgraduate education and research in medical sciences. A significant feature of this period is a proportionately large increase in the output of postgraduates in agriculture, engineering and medicine. The stock of scientific and technical personnel increased rapidly, as Table 3 shows. If the trends continue the estimated stock by the end of 1975 would be about 624,000 graduates in science, 229,000 postgraduates in science, 278,000 graduate engineers, 391,000 diploma engineers and about 117,000 medical doctors

But despite the expansion of facilities for higher education and research, a number of Indian scientific and technical personnel have continued to go abroad over the years. Education and training overseas is a century-old tradition with India's scientific and technical personnel; many qualified people go abroad for further studies, or training, or to acquire wider professional experience. Overseas exposure, especially in a developed country, helps expansion of one's academic, industrial, professional and social experience, and mature students can get particular benefit from it. The post-war period apparently opened up newer and greater opportunities abroad, and as a consequence the number of Indian scientists going abroad during the past 25 years has grown considerably. A good number are going for a first degree, with plans to work for subsequent higher degrees. Opportunities for employment in India have not developed along with the development of educational facilities and this has led a section of the educated unemployed to look for posts outside India. Most return home after a couple of years, some stay longer and others settle permanently in a foreign country. Over the past two and a half decades relatively larger numbers have been staying away for longer periods.

India's scientific and technical personnel abroad have been a matter of concern for some time and have generally found a prominent place in any discussion of the 'brain drain' from developing to developed countries. It is worth considering if all these scientists can be grouped under 'drained brains'. The Colombo Plan Consultative Committee, in its 22nd meeting in New Delhi in October and November, 1972 suggested "the external migration of personnel at the skilled, technical and professional levels for employment abroad under circumstances in which they could otherwise have been employed at once or in the near future, productively at their proper attained level of professional skill in their own country at work of national importance" as a definition of brain drain. From this definition, one might perhaps conclude that the number of Indians abroad constituting a brain drain is small. People planning to come home in the foreseeable future cannot be considered; it is those who have settled abroad more or less permanently and those who have not yet decided their time of return who constitute a potential brain drain.

Table 3 Stock of scientific and technical personnel

	Stock at the end of the year				
	1950	1955	1960	1965	1970
Scientists (graduates)	66,900	114,000	185,000	300,900	480,200
Scientists (postgraduates)	1,000	30,000	51,400	93,400	152,700
Engineers and technologists (graduates)	21,600	37,500	62,200	106,700	185,400
Engineers and technologists (diploma level)	31,500	46,800	75,000	138,900	244,400
Medical personnel (graduates)	18,500	29,000	41,600	60,600	97,800
Medical licentiates	33,000	35,000	34,000	31,000	27,000
Total	188,000	292,700	450,000	731,500	1,187,500

It is difficult to obtain a precise estimate of the amount of India's scientific and technical manpower that is abroad and even more so to collect details of their activities. A special section—the Indians Abroad Section—of the National Register of Scientific and Technical Personnel of India was started in 1958 to gather this information. Indian scientists, engineers, technologists and medical personnel abroad for study, further training or employment are encouraged to enrol in this section of the register. Although registration is voluntary, about 20,000 have enrolled so far (Table 4). About 50% of the registrants have since returned to India, which would leave about 10,000 still abroad. But this figure is believed to be far short of the actual number. On other available data it was estimated in early 1971 that 30,000 qualified Indians were abroad, of which 15,000 were engineers, 9,000 medical doctors and 6,000 scientists.

An analysis of the data in the Indians Abroad Register may be of some interest. About 90% of the 19,824 registrants are from four countries, the USA, the UK, Germany and Canada—the USA accounting for about 38% and the UK about 35%. Among those registered from the USA, scientists constitute the highest proportion (46%) followed by engineers (37%) and medical personnel (12%). Medical personnel and engineers form the highest proportion (39% each) among those registered from the UK, scientists make up 16%. Of the registrants from Germany, engineers are the largest group (63%) followed by scientists (22%); medical personnel constitute only 3%. Fifty per cent of the registrants from Canada are scientists and 33% engineers.

More than 50% of the scientists in the register enrolled from the USA, about 16% from the UK and about 10% from Canada. Among the engineers about 33% are from each of the USA and the UK and about 16% from Germany. About 70% of medical personnel are from the UK and a litle over 20% from the US.

Table 4 Progressive rise in the registration in the Indians Abroad Register

	Science	Engineering	Technology	Medicine	Social science	Management and/or accountancy	Total
1959	678	1,083	145	187	—	215	2,308
1960	975	1,685	280	295	—	270	3,505
1961	1,271	2,287	418	403	—	331	4,710
1962	1,586	2,735	510	583	—	375	5,789
1963	2,070	3,310	636	850	—	432	7,292
1964	2,495	3,945	707	1,175	—	471	8,793
1965	3,006	4,535	800	1,575	13	530	10,459
1966	3,413	5,000	892	1,910	76	552	11,843
1967	3,754	5,279	946	2,135	125	568	12,807
1968	4,048	5,513	988	2,384	154	574	13,661
1969	4,420	5,760	1,020	2,670	188	579	14,637
1970	4,790	6,015	1,050	2,945	239	594	15,633
1971	5,161	6,259	1,083	3,218	266	603	16,590
1972	5,497	6,567	1,107	3,385	305	606	17,467
1973	5,833	6,977	1,136	3,582	338	612	18,478
1974	6,131	7,274	1,171	3,726	351	617	19,270
1975	6,330	7,455	1,188	3,855	374	622	19,824

Table 4 also reveals that the number of Indian scientists enrolling in the Indians Abroad Register is progressively diminishing. Whether this is because of a gradual decrease in opportunities abroad in recent years can only be guessed.

The other interesting fact is that almost all the registrants state their intention of returning to India sooner or later. This obviously brings out the weak side of the register. People who have more or less settled abroad or persons who are undecided about their return do not bother to enrol. Most registrants are on academic assignments but the numbers employed in industry, particularly in production, and those holding senior positions in academic fields are low. A special effort to collect information about such personnel has been initiated recently by the Indian Missions in some developed countries.

India's scientific and technical personnel abroad, properly harnessed, can contribute a great deal. It is, therefore, a question of what can be done to facilitate their return and to use them effectively once they have come back. The usual procedure of recruitment in India, especially to jobs in the public sector, acts as constraint on quick absorption in regular employment. It was, therefore, necessary to evolve a scheme which would ensure temporary placement, at least for those better qualified, on their return from abroad. The Indian government, therefore, instituted in 1959 the 'Scientists Pool' for the temporary employment of well qualified Indian scientists, engineers, technologists and medical personnel, particularly those returning from abroad without a job. Later, social scientists were also brought within the purview of the pool scheme. Persons with high qualifications, who have not been abroad are also considered for the pool—an unorthodox system of temporarily employing and making use of highly qualified people while they look for regular employment through the usual channels. Selections for the pool take place on a continuous basis throughout the year. Everybody enrolled in the Indians Abroad Register is automatically considered for selection near the time of their return, provided they do not have a job in India. The Scientists Pool is operated by the Council of Scientific and Industrial Research at New Delhi.

Those selected are attached to appropriate organisations and receive a salary (actually more of a stipend) from the Council of Scientific and Industrial Research. Any scientist in the pool is free to try for any job in India and leaves the pool on securing one Pool appointments are for a period of one or two years. If a pool scientist is not able to find a regular job within this period, his case is reviewed and the appointment may be extended.

Table 5 gives a breakdown of the number of people in the pool. Up to the end of February 1975, some 10,753 persons had been selected, of whom 9,889 were from the Indians Abroad Register. Of these, 6,163 returned to India and 3,635 of them secured regular employment in India after working for some time as a pool scientist; another 1,070 were able to find a regular job without help from the pool. About 900 other people are believed to have settled in India in regular employment as they did not respond to pool enquiries.

The pool scheme does, however, have its inherent limitations. As pool scientists are only temporary, the user organisations are unable to give them long term assignments. Nevertheless, many pool scientists produce good work and many organisations are keen to obtain scientists, engineers and doctors through the pool system.

An opinion survey of ex-pool scientists, carried out by the CSIR in 1969–70, found that most of them were fully satisfied and nearly all were happy with at least some part of the scheme. There was some dissatisfaction arising from temperamental differences, high expectation, and conflict of interest between the internal staff and the pool scientist. Nevertheless, 46% of the respondents stated that the pool placement gave them confidence to return, 53% felt that it gave a ready working opportunity and 67% appreciated the financial benefit so necessary at the beginning. The pool scheme can, therefore, be considered to have been of considerable help to Indian scientific and technical personnel returning from abroad.

The Indian government also approved a scheme of supernumerary appointments for the quick absorption of outstanding scientists whose services might be useful for research and development projects. The posts are to be specially created for them and salaries appropriate to their background can be offered. The scheme has, however, been used only cautiously so far.

Table 5 Scientists pool

	Science	Engineering	Technology	Medicine	Social science	Total
1959	77	69	26	25	—	197
1960	50	60	15	38	—	163
1961	240	160	26	98	3	527
1962	120	79	28	159	7	393
1963	185	365	37	247	5	839
1964	265	485	37	345	7	1,139
1965	324	321	49	266	8	968
1966	205	162	33	219	10	629
1967	187	156	24	220	20	607
1968	263	113	20	191	12	599
1969	321	167	18	219	18	743
1970	280	151	15	227	9	682
1971	364	215	18	160	14	771
1972	356	369	21	164	27	937
1973	249	245	16	138	10	658
1974	363	253	24	178	19	837
Total	3,849	3,370	407	2,894	169	10,689

Although the schemes of the scientists' pool and supernumerary appointment help those who return to India, they have not proved sufficiently attractive to those undecided about their return or those who have settled abroad. Even though it is not possible to bring back every Indian abroad, some attempt to make use of their talent for specific purposes is worthwhile. Some may visit India for a short time in a professional capacity. Their association with research and development organisations or academic institutions in India may be of mutual interest. Schemes of short term appointment as research associates or visiting scientists have recently been introduced by the Council of Scientific and Industrial Research. People visiting India for a short time can be offered such appointments in national laboratories in cases where their background fits the requirements of the organisations. In the academic field, the universities in India are now in a position to offer short term appointments to Indian scholars abroad during their sabbatical leave. Apart from the immediate gain, such associations provide people with the opportunity to become acquainted with conditions in India and may induce them to stay permanently The Indian government has also recently approved a 'package scheme' to enable Indian scientists and technologists engaged in production or management in industrial organisations abroad to set up manufacturing units in India. They will be helped to secure industrial licences, capital goods clearance and import licences, and if necessary entrepreneurial loans from public financing bodies in India, and to obtain infrastructure facilities such as land, water and power supplies. The Council of Scientific and Industrial Research is the executive agency of the scheme, but its recent introduction means that its impact is yet to be felt

Most of the schemes discussed above have their limitations. Proper and full use of India's vast scientific and technical manpower resources is linked with employment opportunities and appropriate working facilities. The economic growth of a country provides professional opportunities within that country for the scientific and technical personnel. The trained personnel are used in industry, agriculture, research and development and public health to the extent that the economy can absorb. As the growth of the economy in India has not been as expected, this has created the problem of making proper use of India's scientific and technical personnel at home and abroad. Considerable thinking is going on to create effective demand for scientific and technical manpower in viable areas. Emphasis is being placed on schemes which provide greater employment and also on efforts to create employment opportunities in different sectors. It is not necessarily the targets and investment in an economic Plan, but the working strategy, that decides employment potential within the constraints of the given resources. It also calls for a close look at the technology to be used for various developmental projects.

Much employment is likely to be generated for various categories of personnel during India's Fifth Five-year Plan. Employment opportunities for scientific and technical personnel are, however, closely linked with the Science and Technology Plan, an integral part of the Five-year Plan. This is the first time in India that a separate body—the National Committee on Science and Technology—has been set up with the task of formulating a national plan for science and technology. This plan is one of the major policy instruments for achieving self-reliance. It has been prepared with a view to solving pressing problems of agricultural growth, industrialisation, productivity, environmental deterioration and population growth, and gives highest priority to the application of the scientific and technical skill already built up and the development of indigenous technology. The research and development efforts envisaged are expected to open up vast employment opportunities for scientific and technical personnel. It is estimated that about 120,000 jobs will be generated during the implementation of the Science and Technology Plan, of which approximately 10,000 will be for personnel with high qualifications.

Peculiar problems of the British Civil Service

David Davies

Employer of 17,000 scientists, the British Civil Service faces special problems in the motivation and use of its more senior members. Maybe this calls for some fairly radical solutions.

In 1854 two major reports were published on the concept of the Civil Service: Macaulay on the Indian Civil Service and Northcote and Trevelyan on the British Civil Service. In many ways British experience in India influenced the development of the Home Civil Service, and in both services the problems of the early 19th century had been those of nepotism and incompetence. There was talk, in the Northcote–Trevelyan report, of admission to the Civil Service (and this meant the administrative sector) being sought after by the 'unambitious, indolent and incapable'; (see box). The report went on to hint that the lightness of work and security of employment made the Civil Service a natural home for "sickly youths".

The solution to all this according to both Northcote–Trevelyan and Macaulay was to subject applicants to competitive entrance examination. Macaulay spelt out his examination proposals in detail:

English language and literature:	
Composition	500
History	500
General literature	500
	Total 1,500
Greek	750
Latin	750
French	375
German	375
Italian	375
Mathematics, pure and mixed	1,000
Natural sciences	500
Moral sciences	500
Sanscrit	375
Arabic	375
	Total 6,875

"It seems to us probable", he goes on reassuringly "that of the 6,875 marks no-one will ever obtain half". The marking system was simply to ensure equal access to the classical scholar, mathematician and such as the young man proficient in "English, French, Italian, German, geology and chemistry".

The Fulton Committee of 1968, the most recent major investigator of the Home Civil Service observed that the service was still fundamentally the product of the nineteenth century philosophy of Northcote–Trevelyan. From the 1854 reports "emerged the tradition of the 'all-rounder' as he has been called by his champions, or 'amateur' as he has been called by his critics".

In 1854 the government employed practically no scientists and there was no such thing as a national laboratory. And yet the story has its modern aspects. For one thing, the consequent evolution of the generalist administrator as the top dog of the Civil Service rankles with some specialist civil servants (scientists, engineers and so on) whose access to higher management and policy making was (at least when Fulton reported) restricted. For another, the sort of noises that were being made in 1854 about a quiet secluded profession leading to narrow views have been made ever since about civil servants, and as scientists have become civil servants they too have reaped some of this criticism. For instance, in 1931 the Carpenter Report on the Government Scientific Establishments tried to encourage scientists to interchange between research establishments and universities by sharing a common (university-based) pension scheme. (This has now been abandoned.) In 1941, A. V. Hill wrote[1]: "In many of the Departments of government, notably those of the Defence Services, . . . the danger of stagnation and complacency exists". In the 1960's committees chaired by Mott, Sutherland and Zuckerman all in different ways addressed the problem of stagnation within civil service science. In 1972, an OECD report[2] noted that "job security may very well transform agencies in the government sector into havens for second rate scientists who can be assured of making a career there". *Plus ça change.*

> Those who enter it generally do so at an early age, when there has been no opportunity of trying their fitness for business, or forming a trustworthy estimate of their characters and abilities. This to a great extent is the case in other professions also, but those professions supply a corrective which is wanting in the Civil Service, for as a man's success in them depends upon his obtaining and retaining the confidence of the public, and as he is exposed to a sharp competition on the part of his contemporaries, those only can maintain a fair position who possess the requisite amount of ability and industry for the proper discharge of their duties. The able and energetic rise to the top; the dull and inefficient remain at the bottom. In the public establishments, on the contrary, the general rule is that all rise together. After a young man has been once appointed, the public have him for life; and if he is idle or inefficient, provided he does not grossly misconduct himself, we must either submit to have a portion of the public business inefficiently and discreditably performed, or must place the incompetent person on the retired list, with a pension for the rest of his life. The feeling of security which this state of things necessarily engenders tends to encourage indolence, and thereby to depress the character of the Service. Again, those who are admitted into it at an early age are thereby relieved from the necessity of those struggles which for the most part fall to the lot of such as enter upon the open professions; their course is one of quiet, and generally of secluded, performance of routine duties, and they consequently have but limited opportunities of acquiring that varied experience of life which is so important to the development of character.
>
> From the Northcote–Trevelyan Report, 1854.

Who they are, what they do

The British Civil Service employs roughly 700,000 people; about 17,000 of these are in the Science Group and are employed (at least in theory) to practise science. It is difficult to know how many scientists there are in the country at large, but figures for the first destination of university graduates suggest that about one in ten of all recipients of higher degrees in science go into the Civil Service, and one in fifteen of all graduates do likewise.

The scope of the operations of the Scientific Civil Service is

Table 1 Numbers and changes in 1973

	Post (recruitment qualification)	No. (women in brackets)		Salary (provisional, revised April 1975)	Promoted to	Recruited to
Open structure	Under Secretary and above	Includes 85 scientists			11	No data
Other scientific grades	Chief Scientific Officer	32	(0)	£10,950	4	0
	Deputy CSO	200	(0)	*	17	0
	Senior Principal SO	618	(5)	£8,650–£9,798	50	3
Science Group	Principal SO	2,011	(43)	£5,514–£7,205	152	10
	Senior SO (degree and high quality experience)	3,516	(146)	£4,185–£5,778	171	43
	Higher SO (degree and experience)	3,549	(266)	£3,254–£4,454	360	79
	SO (degree)	2,946	(470)	£2,149–£3,565	130	151
	Assistant SO (school examinations)	4,065	(1,220)	£1,276–£2,560	—	563

Source: *Civil Service Statistics* 1973 (HMSO, London).
*Not yet made public.

also wider than that in most other countries, where governmental science requirements are more often contracted out.* There are more than 100 departmental research establishments covering fields as diverse as road research, maintenance of physical standards, forensic science, meteorology, nuclear weapons and the control of plant disease. Sixty per cent of all scientific civil servants work for the Ministry of Defence. For the rest, there is a roughly equal division among Trade and Industry, Agriculture, Agriculture (Scotland) and Environment. Other departments employ smaller numbers of scientists.

An extensive history of civil service science is not called for here, but some landmarks should be noted. Research efforts by and for government (apart from the Greenwich Observatory founded in 1675) could be said to have started with the Geological Survey, founded in 1835 and the National Physical Laboratory, created in 1899. In the twentieth century the Department of Scientific and Industrial Research (set up in 1915) established various further national research laboratories, all of which were advised by boards of independent scientists, industrialists and, later, trade unionists. At the same time defence research stations were also, of course, being established.

During the Second World War there was an enormous growth in the amount of government science done, almost all, obviously, directed to the war effort, and the increasing significance of science to national needs led to the setting up in 1946 of a formalised Scientific Civil Service. Until 1971 the service comprised three classes—Scientific Officer, Experimental Officer and Scientific Assistant, each with its own grading system, but one of the consequences of the Fulton Report on the Civil Service (1968) was a reduction in the number of classes. The formation of the Science Group represented, as Fulton foresaw "abolition of the divisions between higher and lower classes, and a continuous grading system from bottom to top in each occupational group".

Fulton also observed that scientists are 'frequently given neither the full responsibilities and opportunities nor the corresponding authority they ought to have'. It recommended 'more training in management and opportunities for greater responsibility and wider careers'. To an extent this has been done at the very top of the Civil Service where an 'open structure' now exists. Those who reach Under Secretary level (see Table 1) are not separated by specialism; about 10% of these 800 top people are scientists. This is not to say that people at the top can do just anything. Many jobs in the open structure are obviously highly specialised (the three directors at the Meteorological Office are Under Secretaries or above). Nevertheless when a scientist has reached the open structure level it is at least in theory possible for him to move to more general policy-making posts either within or outside science.

The typical British civil servant scientist, however, will not make the top eighty. He or she will have entered with a science degree at the age of 22. He or she will be promoted to Higher Scientific Officer in the late 20s, to Senior Scientific Officer a few years later and to Principal Scientific Officer in the late 30s. The next step may not come at all for the 'typical' person; but in the 40s he (practically no shes now) could take on more administrative responsibility as a Senior Principal Scientific Officer.

He or she will probably work for the Ministry of Defence in a delightful rural setting and never give a moment's thought to the possibility of working elsewhere, either within or outside the service. The chance of his or her getting the sack or being declared redundant is negligible. The facilities are excellent and there is great satisfaction to be derived from the work, which is unlikely to make many demands outside normal working hours. There are few, if any, pressures to submit one's work to any form of external evaluation (particularly if in the classified sphere). Pay is excellent and the government has recently shown itself willing to bend the social contract in giving pay rises. A good pension scheme awaits. Perks on the other hand are poor unless you value a ticket to the Air Show at Farnborough, or being in the ballot for tickets to see the Trooping of the Colour . . .

Behind the word 'typical' lies a sizeable variation in abilities, particularly after Fulton, when the scientific classes were unified. On the one hand there are the former Experimental Officers and Scientific Assistants, who will enter as Assistant Scientific Officers straight from school, and might reasonably hope to rise to Senior Scientific Officers eventually. On the other hand are those who enter only after obtaining 'high quality experience' and who would start as Senior Scientific Officers, with a reasonable hope of making it to SPSO.

Promotion is by appearance before a Board. In the Science Group there is a measure of so-called flexible complementing—an establishment is not defined in terms of the numbers it may employ in each grade but individuals may rise naturally to their most appropriate level. This lack of the 'dead men's shoes' approach to promotion is widely appreciated although one does still sense an obsession with grades in the Scientific Civil Service.

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*Comparisons are complex, however. In any country in which a substantial amount of research and development is done the evolution of government-supported laboratories has followed different pathways. In the UK, the Haldane doctrine (1918) separated off fundamental research from ministerial departments and at present there are large numbers of scientists employed by the research councils on projects inappropriate to individual universities yet without a natural home in a ministry, such as oceanography and experimental high energy physics. There are many others in laboratories supported by the Medical Research Council and based on universities, and there are yet more in the UKAEA. These are sometimes known as public servants as opposed to civil servants: their modes of recruitment terms of service and so on are very similar. Although statistics quoted here do not include public servants, most of the general remarks made are equally applicable to them. In other countries things have evolved differently, particularly where there are federal and state governments. Federally Funded Research and Development Centers in the United States, Max-Planck-Gesellschafts in Germany and the Institut National de la Santé et de la Recherche Medicale in France all sit differently on the fringe of in-house government-sponsored research.

Demands on them

The lot of the civil servant scientist seems at first sight to be a much happier one than that of his colleague in industry or a university. Shielded from the short-term pressure to be productive and from the constant questioning and irreverence of the young, the civil servant can pursue a linear career in which he gains seniority as he gains experience. But does the immense stability of Civil Service science act as a positive hindrance to using scientists' skills in the most effective way? Many would argue that the duties of the civil servant scientist are so different from those of other scientists that it is foolish to apply similar sorts of yardstick—except in salary, of course. There is a lot in this—much of the scientific work is routine, and a large proportion of employees are engaged in routine laboratory and field work and neither have the qualifications for more, nor would seek broader horizons. But the sort of work called for in the name of research is likely to be every bit as demanding as industrial or university research, and the problems to be solved generally much greater, either because they are complex and involve lots of people or because there is some element of a race, military or civil.

The growth of the service since 1931, when it was estimated that there were 1,053 scientific and technical professional people in government employment, has been phenomenal, but figures of Jones[3] show rather clearly that the growth was largely over by 1951. Apart from additions to strength from transfers of United Kingdom Atomic Energy Authority (UKAEA) employees into the service there has been no significant change in the size of the service since 1961.

Table 2 Scientific Civil Service, 1973

Age	No.	Entrants	Leavers
Under 20	654	299	88
20–24	2,280	315	249
25–29	2,358	156	166
30–34	2,054	34	52
35–39	1,801	18	22
40–44	1,958	5	15
45–49	2,301	22	13
50–54	1,706		13
55–59	1,268		21
60 and over	557		188
	16,937	849	827

Source: *Civil Service Statistics, 1973* (HMSO, London).

This is quite a surprise. During this period we have seen immense university expansion, an Election (1964) based on harvesting the white heat of the technological revolution, a growing concern with environmental matters (calling for more governmental intervention), a decline in the popularity of science among students and a major economic crisis resulting in a great reduction in the recruiting of university staff. Through it all the scientific civil service has remained stable in size. One of the consequences of this is a remarkably uniform age distribution of civil servant scientists (Table 2).

Flux—or lack of it

This table also clearly shows some striking facts about the immobility of the profession. An utterly trivial percentage of employees over the age of 30, (by which time some of the junior entrants performing routine tasks will have left), leave before retirement for any reason at all—and practically no new people are hired.

There are routes in for the over-30s. There are a few short-term appointments usually for specific duties. In addition if a senior job falls vacant and there is no appropriate replacement in the establishment, the department may be 'trawled'. If the department produces nothing, the whole Civil Service may be 'trawled'. If again nothing comes forward, recruitment can be from outside by competition. As one research director put it, 'by the time we can advertise on the open market, we've forgotten what we wanted him for'.

There are routes out, too. It is in theory possible to dispense with the services of a scientist who is not contributing, but the procedure is lengthy, and if the scientist will not take the hint and go (and with an excellent pension scheme, why should he?) it is often easier to let things slide than provoke a bloody battle. Whatever management skills senior civil servant scientists may possess, one of them is clearly learning how to make do with the people you have.

If the service does not change in size, how does it manage to adapt to change, and particularly growth, in mission? To a certain extent one suspects that scientists are being called on to work harder than they were ten years ago, say. This of course, is an extremely subjective opinion—everyone always says they are swept off their feet and things never used to be like this. But also to a certain extent the government has to rely on the ability of older scientists to make the necessary adjustments and learn the skills of the 1970s. This is asking a lot: from Table 2 it is clear that nearly half the staff must have joined the service before the transistor and the digital computer were commonplaces. Industry recognises the ageing problem by moving most of its research and development staff on by the age of 35 (this is accepted both by the employer's and employee's side of the civil service[4]). Academic life recognises it by the reverence it attaches to the graduate student and post-doctoral worker. The Civil Service is in a more difficult position because it can do so little about its more senior members and has no real mechanism by which its more junior members have to face the fact that they are not cut out for research and development after, say, a trial period of five years. True there are annual reviews such as every good employer carries out, but although there are some attractive carrots the stick of intellectual ridicule or dismissal is lacking.

Remedies

The Civil Service Department (representing the government) and the Institution of Professional Civil Servants (IPCS) (representing the scientists) do not seem unduly worried by the uniform age distribution. Both point to the different sorts of contribution that can be made by more senior men. The concern is much more obvious among the middle managers—directors of laboratories and leaders of teams. 'My biggest problem?', said one such, having vouchsafed a ten-second interview *en passant* on the stairs, 'Getting rid of all my unproductive PSOs'. Others spelt out in a more leisurely way their worries. There just are not enough administrative-type posts to go around in the laboratories; they would like to see the concept of the golden handshake seriously discussed as a humane and effective way of facing up to the problems of the senior scientist. It is by no means clear that such a radical solution would be resisted by the scientists themselves, many of whom are only too aware that they are marking time but who can find no escape mechanism. With a recent liberalisation of the transferability of pensions, things should be somewhat easier.

There are also other possibilities worth exploring. For years people have talked of increasing the mobility of scientific civil servants, but in general nothing has happened. There is, of course, no such thing as an automatic sabbatical (although intelligent individuals have been able to persuade the management that the future of the Civil Service depended on their secondment for a year to establishments in Australia, California or the South of France). And resistance to even the idea of mobility with a return ticket, such as the Bondi report[5] advocated and attempted to stimulate, is surprisingly strong. The IPCS told me that on the whole they opposed the Bondi conclusions partly out of scepticism that mobility served any generally useful purpose and partly out of concern that exchange schemes put members at a disadvantage by perturbing the normal channels of promotion. For much the same latter reason they were not attracted to the idea of more people coming late into the service. It must be remembered that the IPCS has a fairly wide constituency and although some of the advantages of mobility may seem self-evident to those who have already profited from it, it can be quite clearly seen by others as a threat to the orderly career structure that they had always been led to expect.

On the other hand, the IPCS plans to make an increasing amount of noise in the near future on career prospects for its more senior members. The opportunities for scientists, it asserts, 'do not come within a thousand miles' of those open to administrators. As a start it plans a campaign to get the open structure concept moved further down the service to the Principal level, so the PSOs and above would be indistinguishable, at least in name, from their administrative counterparts. I remain to be convinced, on the basis of the scientists that I met, that there was as much jealousy of the administrators as the IPCS would make out. Scientists on the whole do not join the Civil Service having in mind the administrative prospects it may hold out in later years, and their loyalty tends to remain with science even when the relevance of their skills may wane. Besides, the environment and nature of the work is usually pleasant. Further, the attractions of the flexible complementing system in science are considerable. Nonetheless, although the IPCS may be overstating what its members actually want, it could be fairly near the mark on what they ought to want—a chance to look carefully in their forties (or before) at prospects outside science and yet within government service, for not all would willingly accept a golden handshake.

The dilemma is this: it is no good wanting to keep mobility, late entry and so on away from the Science Group but expecting other parts of the Civil Service to welcome scientists with open arms. For one thing without a loosening up of the rigidity of the structure, scientists cannot be expected to acquire the horizons that will make them seek and welcome new prospects. For another, administrators will say 'you wouldn't loosen up your structure—we are professionals too, why should we loosen up ours?'

Speeding the route to the top

Not all the manpower problems of the Scientific Civil Service are those of motivating the middle-aged. A more pleasant problem is that of spotting the real high flier among the hundreds of scientists who may be in any establishment—and doing it early enough that he or she gets the chance to be effective young. Whenever general comparisons are made between civil services (as for instance Fulton did between British, Swedish, United States and French) unfavourable remarks are made about the age at which people reach the top levels in Britain. In the United States the comparison cannot be a strict one since there is so much greater scope for the 'in-and-outer' scooped up from industry or academic life, given a run of a few years of running something big and then allowed—even encouraged—to move on. But elsewhere it is clear that the remarks are often justified—in the administrative sector in Britain there is often too much of an orderly progression. The Science Group has quite a good record of giving early and major responsibility to people of promise, and directors of major establishments are often appointed well before the age of fifty.

Promotion for the highly talented does not necessarily mean an increase in the administrative burden. Although SPSOs and above normally have rather clearly defined administrative responsibilities, individuals can be promoted to these levels on the basis of their scientific merit alone. It is thus possible to reward the intellectual high flier without asking him to run the show, and about a fifth of promotions at this level are on this basis. Even so there is much agonising over how to move people up quickly, particularly now that a unified class structure means that there is great diversity in those coming up for promotion each year.

Conclusion

The scientific civil service is neither growing nor shrinking at the moment; its stability over the past twenty-five years is probably a combination of the great job security in the Civil Service and a genuine desire not to expand organisations which are difficult to contract at a later date. The perennial complaint against the civil servant is that he is immobile and, lacking anywhere else to go, populates the research laboratory for too long. But what humane solutions are there to this? Maybe the golden handshake. Maybe more middle-level administrative and executive opportunities outside science. For sure these matters cannot be neglected for much longer.

[1] Hill, A. V., *The Ethical Dilemma of Science*, 49 (Rockefeller Institute Press, New York (1960).
[2] *The Research System*, 1 (OECD, Paris, 1972).
[3] Jones, R. V., *Proc. R. Soc.*, A342, 481–490 (1975).
[4] Pay Board; *Civil Service Science Group, Appendix B* (HMSO, London, 1973).
[5] *Interchange of Scientists* (Chairman Bondi, H.), (HMSO, London, 1974).

Scientific manpower forecasting as an aid to planning in Europe

R. E. Woodham

Manpower forecasting as a basis for educational or economic planning in the long term is fraught with problems, but a more important problem on a short time scale is the annual variation in the employment of new graduates by industry, particularly in the UK. Smoothing this out would probably lead to more consistency in the flow of students from schools to science departments in universities.

MOST countries, developed and less developed alike, have experimented with manpower forecasting in one form or another, and their motives for doing so are varied. Broadly speaking, however, the basic reasons for making such predictions are to improve educational planning, to assist in economic planning in the sense of working out the manpower required to produce a certain increase in the gross national product, and to help in vocational planning—by providing the raw material for individual decisions on careers, for example.

Obviously no two countries have quite the same mix of motives for doing manpower forecasting, so the techniques differ, which makes international comparisons difficult. Also, depending on the objective, the forecasting exercise may entail looking at manpower requirements by industries, industrial sectors or fairly small occupational groups such as 'scientists', 'engineers' or 'the highly qualified'. Looking even more closely, one finds that in posing manpower questions words have to be interpreted most carefully: 'requirements' and 'needs', for example, have different connotations—the former implying the manpower required to achieve a target and the latter introducing more of a social element and implying as much a political pronouncement as a forecast—for example, that higher education should be available to everybody rather than on a selective basis.

Planning UK scientific manpower

The acid test of manpower forecasting is its accuracy, yet sometimes that is not easy to gauge because the criteria may themselves be completely determined by the forecasting technique. If you design a questionnaire to find out industry's manpower 'requirements' for example, you get an answer that is uniquely related to the way in which the questions were worded. This can be illustrated by the various forecasting exercises done in the UK for scientists and engineers since the late 1950s. In *The Practice of Manpower Forecasting*[1] (page 240) Gannicott and Blaug examine the many government reports on scientific manpower which emerged in the 1960s and conclude that, on the whole, they placed far too much credibility on their own forecasts. When actual employment fell short of the 'aspirations' of employers, as revealed in questionnaires (they say) the cause was, by definition, put down as an overall shortage.

Table 1 Percentage changes in earnings, for the periods specified, revealed by surveys made by professional institutes in the UK

		Age			
		26–30	36–40	46–50	All workers*
Chemists	1956–59	17.6	18.7	21.2	13.8
	1959–62	16.8	19.4	19.5	17.0
	1962–65	18.4	16.8	17.2	23.5
	1965–68	12.2	9.1	11.4	17.4
	1968–71	25.3	22.7	25.3	33.5
Engineers	1966–68	11.5	11.1	11.9	11.0
	1968–71	25.7	23.5	20.5	33.5
Biologists	1968–71	21.1	23.3	21.2	33.5

* Professional and non-professional.

The pitfalls that attend manpower forecasting, in the case of the highly qualified at least, now seem to have been squarely faced in the UK, and the government no longer sees any need to do further 'requirement surveys', at least on such wide categories as 'scientists', and its whole approach to the collection of statistics (without using them to make forecasts) now seems much more geared to the highly qualified in general than to scientists in particular. After all, scientific manpower forecasting in the 1960s was, as Gannicott and Blaug put it, "a science lobby in action".

It used to be the case (for engineers as recently as seven years ago) that the salaries of scientists and technologists increased at a higher rate than did the remuneration of other workers[2] (see Table 1). In that sense, the position of scientists has long since reached a high point, and has subsequently deteriorated. Even the scientists' 'stature' among the highly qualified—those with at least a first degree or equivalent—has diminished while that of the social scientists, for example, has improved according to this criterion.

Economists would therefore have it that the UK is awash with scientists and that a surfeit of chemists can be traced back to seven or eight years before 1972, a year in which the uptake of graduates of all kinds by industry tailed off so dramatically. (In that year employers recruited some 2% fewer graduates, at a time when 7% more were available; the situation improved in 1973, however, when 15% more posts were on offer to 5% more graduates.)

Table 2 Number of people with university degrees in employment (million)

	Total	Degrees in science and technology	Degrees in science and technology as a percentage
United States	5.170	1.106	21.4
EEC (the Six)	2 175	0.711	32.7
UK	0.662	0.250	37.8
EEC+UK	2.950	0.961	33.9

One result of the 1972 debacle is the phenomenon of the chemistry department with almost as many staff as students—an over-reaction by prospective students to an over-reaction by employers three years before. This is almost certainly a short-term effect obscuring a longer-term trend towards an increase in "qualification density" (the proportion of people within a given group with a given qualification) in Census employment classifications like "Administrators and Managers", which accounted for 10% of all graduates or their equivalent in 1966. The qualification density for technicians went up by leaps and bounds in the 1960s; in 1965 14% of them were graduates, but by 1968 the proportion was 22% (ref. 2). And undoubtedly the long-term trend is still continuing, in spite of indications that at the moment industry is crying out again for the products of almost empty chemistry departments.

Whether or not the increasing qualification density in non-traditional graduate jobs will eventually prove the case for more opportunities in higher education to take broadly based degree courses and for the rapid growth of the polytechnic programme at the expense of the universities, the trends in scientific manpower during the 1960s and early 1970s cast considerable doubt on the accuracy of manpower forecasting.

Europe

From a more international standpoint, the OECD has also come to similar conclusions about the usefulness of manpower forecasting for the highly qualified. The organisation was actively interested in matters concerned with scientists, technologists and engineers (see Table 2) in the late 1960s and early 1970s, and it produced, for example, its famous set of reports on *Gaps in Technology* between member countries, one of which deals extensively with manpower statistics but eschews manpower forecasts. The OECD effort culminated in a conference in Venice in 1972, which concluded that fears about the consequences of overproduction of qualified manpower (overproduction was the *bête noire* at that time) were unfounded on the grounds that it would not be any real hardship for some of the highly qualified to look rather further afield for longer before getting a job. Nevertheless there was a general awareness that it would be helpful to have a better understanding of the relation between the needs of industry for graduates and the output of the higher education system.

Table 3 Swedish industrial planning, 1965

	Increase in industrial output per year (%)	No. of industrial employees	Density of engineers (%)	Forecast no. of engineers
Original forecast	3.5	961,000	4.68	45,000
Revised forecast	6.0	1,074,000	5.85	62,800
Actual no.	–	–	–	53,800

Table 4 Actual and predicted employment of scientific and technical personnel in France, 1954–78 (thousand)

1954 actual	1962 actual	1970 forecast on hypothesis* 1	2	3	4	1978 forecast
460.4	617.8	729.4	868.9	1,009.0	933.5	1,307

* Actual figures for 1970 not available.

There are no moves afoot on the part of OECD members to look again at the question of highly qualified manpower—a fair indication that most countries do not now see it as a problem area—but higher education is going to be examined further from the point of view of both research and teaching. In the latter case, the eventual recommendations are bound to reflect the pressures to widen higher education and increase the scope for more flexible and broadly based courses. The question of university research is likely to prove a more thorny one, however, with countries asking whether universities are losing some of their status as research institutions.

The European Commission in Brussels is also interested in highly qualified manpower, but mainly from the point of view of migration within Europe. A study was launched last year but the outcome is not likely to be clear for a while yet.

Sweden and France

Swedish experience in manpower matters is particularly interesting because Sweden has a relatively small population, has a relatively 'interventionist' Labour Market Board, and in 1969-70 spent 3.7% of total public expenditure on employment services compared with 0.57% for the UK. Here, it seems, is a country which is notionally in a good position to get its sums right. And yet manpower forecasting as a long-term exercise has not been regarded as much of a success there.

An example which seems to typify the situation is a forecast made in 1957 of the number of engineers required in 1965. The results[1] are summarised in Table 3. The calculations were based on a number of premises derived from data for the previous 20 years, in the main involving assumptions such as that the proportion of engineers in the labour force would continue to increase at a rate which could be predicted from the 'historical' behaviour. In the event the projected increase in output was wrong, as was the estimate of the total number of employees (by 10.5%). The estimated number of engineer was thus 18% low, but what is more interesting is that even if the data had been correctly predicted the forecast numbe would have been about the same percentage too high.

In France, manpower forecasting is tied up with economi plans, and a feature of the techniques used is that at the various stages of production of the plan no single model is used opinions and judgement come in at every stage and iteration is allowed to ensure that manpower forecasts are consisten with other economic indicators. A hotch-potch, one migh think, but one in which the straight manpower-requirement approach (setting an economic goal and working towards i in a rather blinkered fashion) is leavened with a little old fashioned experience.

Table 4 shows some salient figures for 1954-78. The fou hypotheses mentioned range from a minimum assumption (1 to a maximum assumption (3). In the hypothesis adopted (4 the occupational structures in various sectors were based on the opinions of the various planners.

Success or failure?

Although the present techniques of manpower forecasting leave much to be desired as far as predictions in the long term are concerned, over shorter periods of two or three year better results ought to be possible. Unfortunately short-term forecasts that are updated and corrected every year, say, are o relatively little use in educational decision making because o lags built into the education process. On the other hand accurate short term forecasts could form the basis of a continuou rather than a sporadic source of manpower data; in addition more needs to be learned about the relationship between th requirements of industry for graduates and the output of th educational system. These could be used as the basis of ongoin policy modifications—if indeed the country concerned actuall has a manpower policy at all—or, more important, as the firs step towards persuading industry that a severe cut in th number of highly qualified people taken on in one year, followe by a huge demand a year or so later, is of no advantage to it i the slightly longer term and unnecessarily disruptive of th educational system back through the universities and into th schools.

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[1] *The Practice of Manpower Forecasting* (edit. by Ahamad, B., and Blaug, M. (Elsevier, Amsterdam, 1973)

[2] *Employment Prospects for the Highly Qualified*, Manpower Paper No. 8 (HMSO London, 1974).

Training for British participation in the North Sea

Eleanor Lawrence

Specialised graduates in the fields of petroleum geology, geophysics and petroleum engineering will be needed to man Britain's participation in the North Sea oil industry. Three universities in Britain have been identified as the centres for the necessary training effort. This article reviews the progress made so far.

TWELVE months ago, the government directed the University Grants Committee to set aside some £300,000 over the next 3 years to the end of the quinquennium, specifically to finance petroleum-related courses in three British universities, Imperial College in London, and Heriot-Watt and Aberdeen in Scotland. This unprecedented commandeering of UGC money for a specific purpose followed a 1973 report by a working party of the Training Services Agency (a department of the Manpower Services Commission) on Education and Training for Offshore Development, which identified possible shortfalls in the numbers of trained British scientists (geologists and geophysicists) and engineers available to work in the North Sea oilfields.

In the discussion on possible shortfalls, the distinction must be drawn at the outset between non-specialist geologists, geophysicists and engineers, and those with an academic trainin specifically directed not only towards the theoretical aspects o petroleum studies but also towards the sort of practica problems they will have to solve in industry itself.

There is certainly no shortage of geologists *per se*—man people see an overproduction—but petroleum geologists o explorationists are rare—(especially those with a practicall orientated training). Engineers graduating with a first or even masters degree in petroleum engineering also form a relativel small proportion of the total engineering output, but in th case there is also a growing shortage of good engineers of an persuasion.

In the past, the lack of specialised training did not seem t pose many problems. At the time, the large oil companies o Britain's doorstep, Shell and BP, preferred to take non-specia lists and train them within the company through their extensive well-established training schemes. What specialised course there were at British universities either disappeared, as did th petroleum engineering course at Birmingham, or survived b taking in a large proportion of foreign students. But with th increased activity in the North Sea, the situation has change somewhat. Many of the companies operating in the North Se are essentially foreign-based—American companies wishing to replace their American personnel with British graduates and companies operating far away from home-based training schemes, with limited on-the-spot facilities, are now in the market for both specialised graduates and post-experience training facilities in petroleum geology and engineering. Small British oil companies such as those constituting Brindex will need considerable numbers of exploration geologists, production geologists and engineers if they are to help exploit North Sea oil.

The apparent shortage of British petroleum engineers and geologists to work in the North Sea is not, however, simply one of under-production. Imperial College, for instance, has been running oil technology courses in its geology department in the Royal School of Mines since 1913 and there are many highly qualified and experienced British geologists and engineers in the oil industry throughout the world. One of the problems, according to Professor Colin Wall, the head of the newly-established Section of Petroleum Engineering at Imperial College, is that conditions of employment for competent qualified petroleum engineers are much better abroad where the tax position is far more favourable. So the training effort must be adjusted to take account of this wastage. Imperial College and Heriot-Watt have been designated as centres of petroleum engineering and Aberdeen as a centre of petroleum geology. Professor Arthur Whiteman, the new head of the section at Aberdeen stresses that they are concerned not simply with petroleum geology but with the problems of petroleum exploration geology and geophysics, a very different approach. Petroleum geology as taught as a component of many geology courses tends to concentrate rather on the academic and theoretical aspects and certainly does not provide the applied viewpoint which an exploration geologist working for an oil company, or for one of the independent contractors, will need.

In all the centres the one-year MSc course has been chosen as the main academic training vehicle, although undergraduate courses are being or will be offered as well. But the general view is that a one-year course offered to slightly older students who are committed to a career in the oil industry will produce better results more quickly. Heriot-Watt's new MSc course in petroleum engineering for about 20 students is due to start this year. It will be taken within the Institute of Offshore Engineering, which was established in 1972 with a grant from the Wolfson Foundation for training and research in all aspects of constructional engineering for the North Sea. As well as the MSc in petroleum engineering, the Institute will offer an undergraduate course in Offshore Engineering (the first students will start the full 4-yr course this year; it has been offered as a final year option for the past few years). But in the short term, probably the most valuable contribution the centres can make, is in the short post-experience courses they offer to staff already employed in the industry. Imperial College has run two such 10 day courses already, for a total of 21 people and hopes eventually to cope with about 50–60 students of this type each year. Already the British Gas Corporation, Conoco, Core Labs, the Department of Energy and Shell (UK) Expro have made use of these courses. The Section of Petroleum Geology will also be running such courses shortly.

Even when full-time courses are provided, it does not automatically follow that it is easy to find British students to fill them. On Imperial College's MSc course in petroleum engineering for 1974–75, only 5 of the fifteen students were British. The remainder came mostly from oil-producing states such as the Middle-East countries, Nigeria and Australia, financed by oil companies operating in these countries.

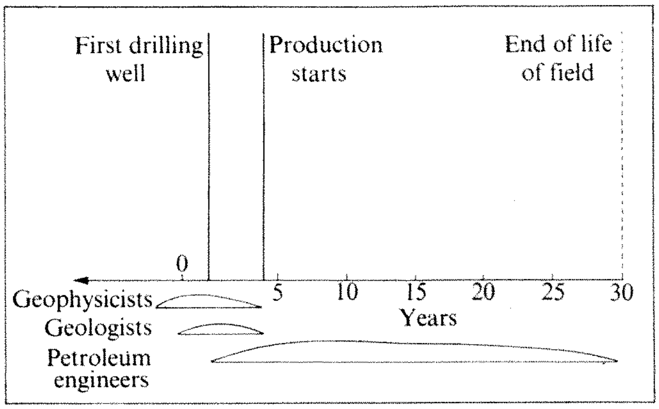
The situation is different for undergraduate students, most of whom are British. This imbalance arises in great measure from the difficulty which British students find in obtaining grants to do postgraduate work, a situation which is unlikely to change without special help. The Geology Department at Imperial College has no shortage of applicants for its long-established MSc in petroleum geology (which has not been allocated special UGC funds). This year only about one in every five applicants will get a Natural Environment Research Council grant. British students can no longer afford to study at British universities and the vacant places will be filled by students from the Middle East on petro-dollar supported grants. Even when studentships are available, as at the new course at Aberdeen, there is still difficulty in attracting students. Professor Whiteman considers it hard to attract students to a grant-aided course, because at the moment a good first-degree graduate can join an oil company and be paid between £2,000 and £3,000 within his first year.

This situation may be changing, however, as the downturn in straight oil exploration activity this year may mean fewer opportunities for the non-specialist. This downturn has also been experienced by the Geology Department at Imperial College which saw five out of nine oil companies cancel the routine 'milk-round' interviews this year. But the picture could change almost overnight. The downturn has been variously attributed to the stabilised price of oil, the British Government's policies of participation and the proposed Petroleum Revenue Tax. If circumstances change there is still a great deal of worthwhile exploration to be done both in the North Sea and off the western coasts of Britain and Ireland.

These factors highlight the difficulty of forecasting manpower requirements in this field, especially when the personnel take at least three or four years to train through a first degree course followed, perhaps, by a specialised masters degree. In the life of an oilfield, different qualifications are needed at different times (Fig. 1). The survey geophysicists and geologists come in first, exploring for structures which might contain oil. When the first exploration well is drilled, the production geologists and the petroleum engineers must decide whether it is worth developing; hundreds of millions of pounds can rest on their interpretation of the results from a few tests on the one well. Petroleum engineers are then needed throughout the life of the oilfield, but the geological and geophysical exploration effort slackens off on that particular field—perhaps less quickly than usual in the North Sea, where conditions are novel and sometimes unstable. Reservoir or production geologists are frequently needed in the development and production phases of an oilfield.

In the present round of North Sea development the peak requirement for geologists and geophysicists has now been reached. Their main field of operation will now move to the western basins (Celtic Sea and West Coast of Scotland), although another round of licensing in the North Sea could lead to an upswing of activity there. Shell (UK) Exploration built up over the past ten years from 0–66 petroleum engineers, 0–43 geologists and 0–25 geophysicists. The demand for geologists and geophysicists has now reached its peak and the present numbers will be kept steady over the next two years

Fig. 1 Diagram indicating qualitative manpower requirements during the life of an hypothetical oilfield. Production geologists are also required later on in the life of the oilfield.



with a recruitment of about four people in these categories each year to keep successions moving; but a further 15 petroleum engineers will be required.

Courses were not ready to meet the sudden demand for specialised geologists and geophysicists needed for the original North Sea oil breakthrough, because it was simply not foreseeable in time. The Aberdeen MSc course in petroleum exploration studies, now in its second year, is producing five students each year, which could be increased to ten if suitable candidates could be found. With increased financial support over and above the £15,000 per annum provided by the UGC for the three years from 1974, 20 students could be accommodated, but a capital grant for equipment and buildings has not yet been forthcoming although negotiations have been opened with the Department of Energy for more funds.

About 20 students each year take the MSc in petroleum geology which Imperial College has offered for many years; Dr R. C. Selley of the petroleum geology section estimated that a production of about 50 from all UK sources each year could be absorbed into the industry if the economic conditions were right and exploration activity was on the upswing again—this also allows for those going overseas to work.

Although the situation does not look particularly promising for geologists and geophysicists at the moment, the training efforts must be started and sustained now, otherwise Britain is going to be unable to supply the necessary specialist manpower for the next round in exploration. Nobody has yet any idea what range of exploration will be needed. At present, operations around British coasts are confined to the relatively shallow continental shelf. Eventually, the effort may move out into the deepwater Atlantic, off Rockall and off the west coasts of Scotland and Ireland and into more difficult areas of the North Sea where new "plays" must be developed.

One of the few attempts to estimate the numbers of qualified scientists and engineers needed for the North Sea operations up to 1980 was made by the Manpower Services Commission in the course of a report dealing with the whole problem of manpower for North Sea oil and related industries, published early this year. Their estimates were based on figures supplied by the Petroleum Industries Training Board, who took the numbers of geologists/geophysicists/engineers needed to man each seismic survey vessel, drilling rig or platform, and then projected these figures on estimates of exploration, drilling and production activity up to 1980. But this sort of forecast can be notoriously inaccurate as activity changes with the economic wind.

Table 1 Estimates of some selected categories of manpower needed for offshore operations in the North Sea

	1973	1976	1980
Professional grades (total) of whom:	1,315	1,785	2,320
Geologist/geophysicist	440	485	520
Petroleum engineer	190	280	330
Production engineer	210	320	440
Drilling engineer/toolpusher	240	370	465

1973 figures are actual survey figures collected from the oil companies. These figures relate only to men actually employed on offshore operations. The MSc training courses in petroleum engineering will produce graduates qualified as both production and petroleum engineers in the categories above and the petroleum exploration studies course will provide both geologists and geophysicists.

A sounder basis perhaps for forward planning, in the estimation of one North Sea watcher, would be a comprehensive census by category and nationality of all scientists and engineers working at present, not only in the field but in the UK headquarters of the companies. Extrapolation of trends such as the 'de-Americanisation' of the American-based companies operating in the North Sea and natural wastage could be built into the model. The opportunities for British graduates could then be pinpointed for at least some years ahead and the training programmes adjusted appropriately.

Britain's need for scientists and engineers qualified in petroleum studies is not simply restricted to the North Sea operations of the oil companies. The Department of Energy, the Scottish Development Department and the Offshore Supplies Office, for example, all need qualified, experienced expertise if they are to direct Britain's participation in the oil boom intelligently.

Some recent estimates put the number of those with technical (as opposed to economic) qualifications in oil-related topics in the Department of Energy, at about 30. The Offshore Supplies Office has a total staff of 90 but very few technical specialists, and there is a general lack of industrial experience. At the Institute of Geological Sciences, of a total staff count of around 1,000, about 60 are concerned with the geology of the North Sea as it relates to oil and of these, only about 40 are directly concerned with the actual geophysical and geological data coming out of the North Sea through the oil companies.

The Gas Council has been involved in North Sea operations for a long time now and has a good exploration department including around 20 scientists and engineers; but the National Coal Board, whose interests in the North Sea will be taken over by the proposed British National Oil Corporation, has an oil department of 13 including only three people with direct exploration and other technical expertise. The British National Oil Corporation will also require qualified manpower soon. If this company is to develop into a fully-fledged working oil company and not simply the state holding company, British technical specialists will be needed urgently.

Some idea of the scale of manpower needed in the operation of a large oil company is given by BP's figures for its North Sea operations. It employs around 350 technically qualified professional staff at its UK Continental Shelf Offices with a back-up of some 145 technical staff in London. Its Forties Field operation alone employs 50 technical staff plus 100 support staff at headquarters. Conoco's exploration and production division in the United Kingdom employs about 330 technically-qualified staff.

So although the North Sea operation itself is hardly likely to collapse through lack of manpower, if Britain wants to build up a body of qualified, experienced oil men within the United Kingdom, the training programme is very necessary although the blockage is likely to occur even before entry to the specialised training courses. The oil industry seems to be unattractive to young people, according to Professor Wall. They see it as a foreign-dominated, multinational giant. Coupled with the general swing away from science and industry apparent among school-leavers, this is probably the greatest threat to Britain's full participation in the oil industry now on her doorstep.

Use of manpower in Japan

from a Special Correspondent

Because of the unique structure of Japanese society, discussions about needs for qualified manpower have only recently started.

BROADLY speaking, the way Japan uses her scientific manpower is unique because three crucial concepts are involved: one can call them cooperation, security and self-help.

The first embodies the fact that the boundary between the public and private sectors is more blurred in Japan than elsewhere. Japanese society, as is well known, does not encourage individualism and, moreover, industrialisation in the 19th century was the result not only of private enterprise but of deliberate government policy. Another feature, perhaps a result of all this, is that there has been very little public debate about the need for trained people. This situation is now changing, at least partly because the slow-down in the growth of the economy is causing problems. The outcome of the debate is uncertain, but it will certainly be a consensus of the views of universities and public and private interests. It would be an impossible, and meaningless, task to say which particular idea came from where.

The second refers to the security of jobs in Japan, which is usual at all levels of society. Mid-career transfers between sectors, or even between companies, are rare. Within each organisation, whether company, ministry or university, promotion is commonly determined far more by seniority and far less by merit than in the West, and it is loyalty rather than the prospect of promotion that provides the incentive to work.

The third concerns the extraordinary diligence and academic ambition of Japanese schoolchildren and their parents. Although fees are charged for schooling above the statutory minimum school-leaving age, 90% of children do stay on and a third of them go at great expense to a university.

Since the war Japanese education has been organised along the lines of the American framework. Standards, on the other hand, seem to be much more European and university entrance papers are much more reminiscent of British scholarship papers. For example, schoolchildren intending to study science learn differential calculus as far as simple differential equations, while still at school. (This is in spite of two handicaps under which all Japanese children labour: first, literacy in their own language entails the memorisation of at least 2,000 Chinese characters each with at least two pronunciations, and they have to be able to read and write English, which is taught to an extremely high standard but rather formally as though it were a dead language).

The boom in university education has produced an immense number of private universities. These are no entrants' first choice, for they lack the reputation of the older state universities and are much more expensive. The boom began in 1967 when 26 universities and 55 junior colleges were established. There are now 304 four-year private universities, 434 two-year private universities and seven private technical colleges. Altogether they make up about 82% of colleges and universities in Japan.

There is now much concern about the quality of these institutions and, it has to be admitted, about the quality of the students they accept. The decline in the proportion of students wishing to specialise in science has been greeted with private relief in some government circles. A White Paper published last year emphasises the need to establish close cooperative relations between government and existing institutions to coordinate innovations. The fact is that up to the present time there has been no study made on the question of how many high school graduates should go on to university.

In the middle of March 1975 the Ministry of Education published an ordinance revising the criteria which a proposed institution must fulfil in order to be approved. This is, however, only a step towards more concerted coordination. The new criteria are (1) geographical location—the ordinance seeks to halt the centralisation of universities in Tokyo Metropolis and the three neighbouring prefectures, and (2) financial fitness. The background to the latter provision illustrates one of the problems of a 'lobby group' style of government. Medical and dental schools traditionally raise capital by requesting donations from applicants for admission. It seems to be the present intention to discourage this practice by removing the need for it. This is partly the result of widespread allegations

Table 1 Holders of higher degrees from Japanese universities (1972)

		Doctor's degree				
Subject	Master's degree (two years post-graduate)	Old system*	National universities†	Public colleges†	Private universities†	Total
Literature	20,032	1,636	381 (319)	14 (5)	249 (213)	644 (537)
Education	2,851	—	167 (116)	—	1	168 (116)
Theology	1,245	3	—	—	10 (5)	10 (5)
Fine Arts	1,507	—	—	—	—	—
Social sciences (including law, economics and commerce)	19,495	1,598	501 (324)	32 (28)	283 (187)	816 (539)
Science	15,881	5,830	5,513 (2,559)	397 (302)	182 (84)	6,092 (2,945)
Medical science	3,683	70,590	18,338 (9,358)	3,314 (1,823)	7,801 (4,611)	29,453 (15,792)
Engineering	39,960	6,715	5,562 (2,784)	307 (179)	557 (308)	6,426 (3,271)
Agriculture	7,386	3,287	2,718 (1,686)	45 (20)	145 (105)	2,908 (1,811)
Veterinary medicine	459	116	60 (41)	—	117 (92)	177 (133)
Fisheries	392	82	67 (48)	—	—	67 (48)
Total	112,891	89,857	33,307 (17,235)	4,109 (2,357)	9,345 (5,605)	46,761 (25,197)

*Old system and †new system: before 1945 doctorates were awarded by the German system. Since then they have been awarded either by examination including a thesis after three year post–master's degree attendance at university or by a thesis only extra-murally. Total numbers awarded are shown with the latter method in brackets.

that this practice has led to unfairness in selection, and even in success in final examinations. These allegations have represented many people's private opinions for several years—it is one of those things that 'everybody' knows but has only recently come to the surface. A headline in the *Mainchi News* of April 5, 1975 reads "30 million yen not enough" and the newspaper alleged that medical examinations at a leading university are completely rigged and that the going rate for entering this lucrative profession has now passed 30 millon yen (about $100,000). The power of the medical lobby is seen, incidentally, in another way: doctors and dentists are allowed by a widely resented law to claim up to 90% of their gross fees as expenses and so pay no tax on incomes of typically 10 million yen.

Whatever the truth of that, non-medical graduates have the usual options of staying on for higher degrees or joining government or industry. Table 1 indicates the numbers who can and do opt for the first alternative. The difference between Japan and the rest of the world is that if the present system of 'life-long hiring', as it is called, survives the slow-down in economic growth that has sustained it for nearly a century, then the decision will continue to be a commitment for life. The system has come under strain in the past year. A distinguished professor of industrial sociology, Hiroshi Hazama, defended the system recently in *Chuo koron* magazine on the grounds that the extraordinarily high population density of Japan makes American-style competitiveness exceedingly undesirable, for it would raise stress to an intolerable level. He criticised the few companies which have introduced this policy in the last decade. The lack of competitiveness in the private sector probably accounts for the fact that it enjoys in graduates' minds neither less prestige than the public sector, as in Britain, nor more as in the USA. And certainly there is no sign of competitiveness in the public sector. Not only does the government pay all its scientists the same; it pays them the same as lawyers and economists.

Israel at the crossroads

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A small country engrossed in the problems of immigrant absorption, Israel could either limit its responsibility for the growth of her exploding academic personnel, or risk a breakthrough in a new kind of scientific manpower.

WHEN Jewish pioneers left Eastern Europe in the 1920s and 30s and immigrated to Israel, they sang the then most popular song "We've come to this land to work it and to be redeemed". Most of them had forsaken well-to-do homes and were members of various Socialist–Zionist Labour Movements. Through this song they tried to express their desire to cut themselves off from the traditional Jewish scholarly philosophy which despises physical labour. In this ancient-new land of theirs they wanted to recreate a new brand of Jew, who works on the land and engages in hard physical work. They succeeded in building Kibbutzim, villages and towns; they developed modern agriculture and industry, but they could not detach themselves entirely from their past.

The first big wave of modern Jewish immigration to Israel came after the First World War. When these pioneers arrived in Palestine they did not find a large Jewish population nor did they find a Jewish State. But there were already two academic institutions; the Technion in Haifa and the Hebrew University of Jerusalem; the first laid its foundation stone in 1912 and opened its gates to students in 1924 and the second in 1918 and 1925 respectively. Thus the history of scientific manpower in Israel preceded the establishment of the State of Israel by about a generation. The Jewish population of Palestine in 1925 numbered about 122,000 out of a total population of 800,000.

Outstanding position in science

Israel is one of the smallest countries in the world, but it occupies a disproportionate place on the scientific map in relation to its physical size. At the end of 1973 the population of Israel was about 3.3 million, of whom 2.81 million (85%) were Jews (about 19% of the total number of Jews in the world).

In 1973 the civil working force of the country comprised more than one-third of the total population and numbered 1.088 million. Of the total working force, 41.8% were employed in industry including construction and communications; 37.4% in public and private services; 12.7% in commerce; 7.3% in agriculture and fishing. The numbers employed in agriculture have been decreasing slowly but steadily from 17.5% in 1955 to 7.3% in 1973. The number of professional, scientific, technical and related workers in 1973 was about 17% compared with about 16% in 1972 and 11.3% in 1960. The expansion of industry, construction and the Civil Service was accompanied by a process of professionalisation in many occupations. Israel has the largest proportion of professional, scientific, technical and other related workers in relation to its total population and the second largest relative number of people with academic degrees in the Western World.

Of the greatest interest, are the various factors responsible for the rapid growth of scientific and technological manpower in Israel, namely the number of scientists and engineers engaged in research and development (Table 1), the development of higher education in Israel and the 'brain gain' through immigrating professional personnel. It is also interesting to note the very high student population ratio in Israel (second only to the United States and Canada) which indicates an intensive academic programme, and the relatively high investment in research and development (Table 2).

Higher education

From their onset the institutions of higher learning in Israel have established very high academic standards. Today there are seven universities in the country which grant degrees at all levels, PhD included. All put strong emphasis on research.

Table 1 Scientists and engineers in civil research and development in Israel

Sector	Total	Full-time equivalent
Total	5,400 (est. 1974)	3,350
Government	800	700
Institutes of higher learning	3,300	2,000
Industry	1,300	650

Preliminary estimates. Sources: National Council for Research and Development, Prime Minister's Office, Jerusalem; Central Bureau of Statistics, Jerusalem.

Table 2 Allocations for research and development in the civilian sector

	Source of Funds (1973)				
Sector	Total	Government	Institutes of higher learning	Industry	Hospitals
Total	507	297	128	75	7
Government*	88	86	—	2	—
Institutes of higher learning	314	185	128	1	—
Industry	95	23	—	72	—
Hospitals	10	3	—	—	7

Preliminary estimates in millions of Israeli lira.

*This sector covers almost all of the research institutes in Israel.

Sources as in Table 1.

Government budget, financial year 1973/74 (IL million)	19,800
Total GNP, 1972 (IL million)	28,958
Gross output value in industry, 1972 (IL million)	17,302
Government allocations for research and development	
(1) Civil research and development expenditures in natural sciences and engineering, 1973 (IL million)	507
(2) Civil research and development expenditures in industry, 1973 (IL million)	95
(3) Civil research and development expenditures in industry as percentage of gross output in industry, 1972	0.55
(4) Research and development expenditures in universities, 1973 (IL million)	314

Source: ref. 2.

These institutes also serve as major links between Israel and the world Jewish community by making Israel a cultural centre of world Jewry and by attracting students and scholars from abroad.

Forty per cent of the 50,000 students and graduates in 1973 studied science and mathematics, engineering, agriculture and medicine. The accelerated growth of higher education has been caused by the rapid growth of intermediate education. Recently, pressure from school-leavers has caused a reaction in the universities reminiscent of the development in Europe, where the expansion of Social Sciences and Humanities faculties has occurred.

The academic system in Israel is the major supplier of manpower for research, academic teaching and other scientific or technological employment in the country in spite of the large 'brain gain'. During the past few years about 6,000 students graduated annually from the academic institutions and about 4,000–4,500 academicians came into the country; thus the academic population of Israel increases annually by about 10% (Fig. 1).

In addition to the rapid expansion of academic education there occurred recently in Israel a rapid development of semi-academic education, particularly in teacher training, but also in technical and other professional fields. In 1973 there were about 21,000 pupils in post-intermediate education comprising courses in teacher training, practical engineering, technical work, and paramedical occupations.

Israeli universities are autonomous and independent institutions. But they receive over 80% of their operating funds from the public, be it the Government, the Jewish Agency or support from abroad.

Dynamics of research and development

Table 3 reveals the very rapid growth of the scientific establishment in Israel. National emphasis has been put on basic research, which is considered to be "elite" research. This attitude has its roots in the traditional Jewish approach to learning and it has been responsible for the fact that over 60% of the non-defence research conducted in Israel is performed in academic institutions. The assumption has been that science is instrumental for technological and industrial advance. Therefore, university science should be a major factor in starting off technological innovation and promoting the growth of science-based industry. This emphasis on academic learning has resulted in the neglect of applied research both in the universities and in government research institutes. Dr S. Wald has written that "Israel appears to be the last bulwark of the old faith: an attitude which puts theory and pure science above practice and application has until very recently pervaded everything in the civilian sector, Industry, University, Government and public opinion"[1].

Israel's culture is predominantly European. Therefore the economy demands highly skilled and educated personnel. In the absence of important raw materials it is clear that future economic growth will have to come from those industrial sectors which need skills, engineering and applied research. But the high rate of government allocations to basic research, the inability of local industry to undertake sizeable research and development of its own, coupled with the tendency of universities to shun cooperation with industry and applied research, underline the special responsibility of the government in Israel towards development.

The percentage of the gross national product which is allocated to all forms of research and development is very high in Israel (2.4%; second only to the United States). Table 4 illustrates the very high government participation in funding research and development in Israel.

The rate of government allocations to university research and development in relation to its allocations to other sectors is very high (59%); government participation is lower in most western countries and only in Japan it is slightly higher. Table 2 shows how small is the scope of industrial research in Israel compared with university research.

Between 1969 and 1972 most of the academic graduates were absorbed in the universities, as researchers or teachers. During those years, the growth rate of universities in Israel exceeded the growth rate of intermediate education. The high value placed on pure science has had other consequences; the relatively low status of engineers compared with scientists, the lower status of industrial employment compared with university employment, and a greater attraction to pure science, mathematics and medicine rather than engineering. These facts coupled with the relatively small size of Israeli industry, mean that industry will not indulge in a large research and development programme nor employ a large number of academic and technical personnel. And last but not least, until several years ago the universities were reluctant to get involved in applied research and they are still slow in developing intellectual and practical links with industry. It is characteristic of industrial countries that their industry is able to finance its own research and development. Whereas the value of industrial

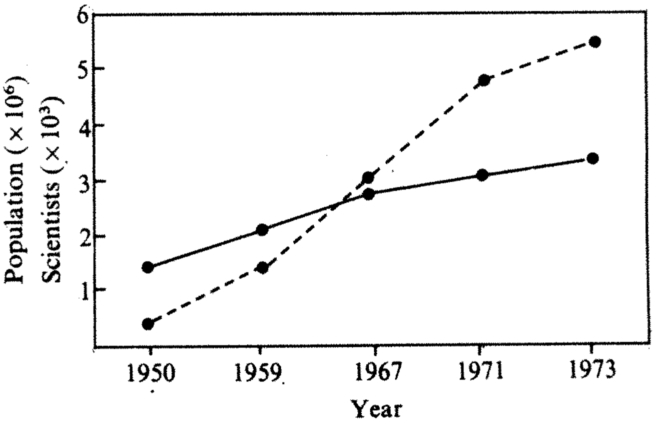

Fig. 1 Comparative growth of population and scientists in Israel. ———, Population; – – – –, scientists.

Table 3 Growth of science in Israel, civilian sector

	1950	1959	1967	1971	1973	Rate of growth (%) 1950–73
Total population (in thousands)	1,810	2,000	2,660	3,095	3,307	2.8
Scientists and engineers engaged in civil research and development	400	1,400	3,000	4,800	5,400	13.5
Research institutes	15	32	54	60		
Academic institutes	2	5	7	7	7	3.5
Students	1,800	9,800	28,000	40,000	50,000	27.7
PhD degrees in natural sciences, agriculture and engineering (Medicine excluded)	10	91	123	236	277	27.7
Allocations* for civilian and university research and development			128	200	507	

*In millions of Israeli lira.
Sources: refs 3 and 4.

production against gross national product in Israel is now approaching the ratio obtaining in developed industrial countries, total national allotment to research and development in industry as a percentage of total national allotment to civil research and development is more than three times lower in Israel than in developed countries. This situation places Israel nearer to the group of developing countries than to the developed ones.

Why a central scientific manpower policy?

The assumption that rapid economic and industrial growth is correlated with accelerating technological development is deeply rooted in Israel. But the expansion and rapid development which prevailed in the country after the 1967 war has been fading since 1972. The world economic crisis has drastically diminished foreign investment and financial help to academic institutions. There have been signs of latent unemployment among professional persons. The government is being forced to take a stand and pursue a central policy of scientific manpower as a result of its heavy commitments to research and development and because of the approaching crisis in the scientific manpower labour market. The institutions of higher learning annually produce about 300 PhDs, thus increasing their number by about 5% every year; in addition one has to consider thousands of Israeli academics abroad, 500 of whom try to return to Israel each year, and the immigrant 'brain gain'.

There has been a positive development in government approach to the problem. The Ministries of Defence and Agriculture have been alert to the situation for some time; other ministries are in the process of creating the tools to cope with it. The National Council for Research and Development (NCRD) has crystallised the two basic inbred weaknesses of the scientific situation in Israel; the imbalance between the efforts of basic research and those of applied research and technological development; and the imbalance between available good scientific and technological manpower and its use in suitable jobs. NCRD has also outlined two primary missions which the government has been advised to put top of its list of priorities within the framework of the national science and technology policy. The government should intensify applied research and development efforts and enhance their quality, to turn them into a valuable tool to aid the solution of economic as well as absorption and social problems; and it should coordinate all operational schemes and investments in science, technology and higher education in Israel, to achieve a proper relationship between the creation of knowledge and production of scientists as well as between the use of this knowledge and the creation of suitable jobs for the pool of scientific manpower.

Table 4 Government allocations in research and development (including Social Sciences), as percentage, of total expenditure in research and development

	Government participation in funding total research and development: civil and military	Government participation in funding civil research and development	Military research share of total government allocations (%)
Norway (1970)	58	56	6
Canada (1970)	54	51	11
France (1972)	50	42	28
Italy (1971)	41	40	4
W. Germany (1971)	39	35	15
Netherlands (1972)	38	37	4
Japan (1969)	14	14	2
Israel (1973)	>70	59	>40

OECD data, excluding Israel.

To tackle these missions, the Israeli Government has set up a Ministerial Committee for Science and Technology and has also set up interim bodies to assist in solving operational problems in the various ministries; such bodies are the offices of Chief Ministerial Scientists, various advisory and executive agencies, the University Grants Committees, as well as various scientific services, such as the Center of Scientific and Technological Information and the Center for Absorption in Science. Operating such bodies requires a certain budget, and the government is preparing a National Budget for Science.

In this context it is important to note a new trend which has been pursued in the universities, on the initiative of the Center for Absorption in Science, with the aim of searching for new organisational forms to absorb scientists. These are scientific groups which aim at developing the nuclei of research organisations, production companies and various services based on research and development. These groups will liaise between universities and industrial centres, science-based industrial parks near universities, research institutes and new research and development groups (with many new immigrants). These bodies have been designated to explore new scientific and technological fields worth developing, without any commitment to maintain them or their personnel after their term expires.

Thus there is a national urge to support and promote science. But there is an obvious lack of precise definition as to why such support is good for the country; and there is a lack of a central research and development policy, making the best use of available manpower. Academic manpower can be a great force when properly used.

Great expectations

The era of sporadic development is over. Both the academic establishment and government officials have come to realise that a crisis is possible not only among blue-collar workers but among academics as well. When such a crisis occurs it is necessary to reconsider which are the best employment alternatives for the available and projected personnel.

It seems that recent events have placed two alternatives before the government: either to let developments take their course, or else the government should face the challenge and use its enormous budgetary and tax measures to pursue a central scientific policy, which will focus on the academic manpower of the country and exploit its vast potential. If the intelligence and intellectual imagination of our people is the

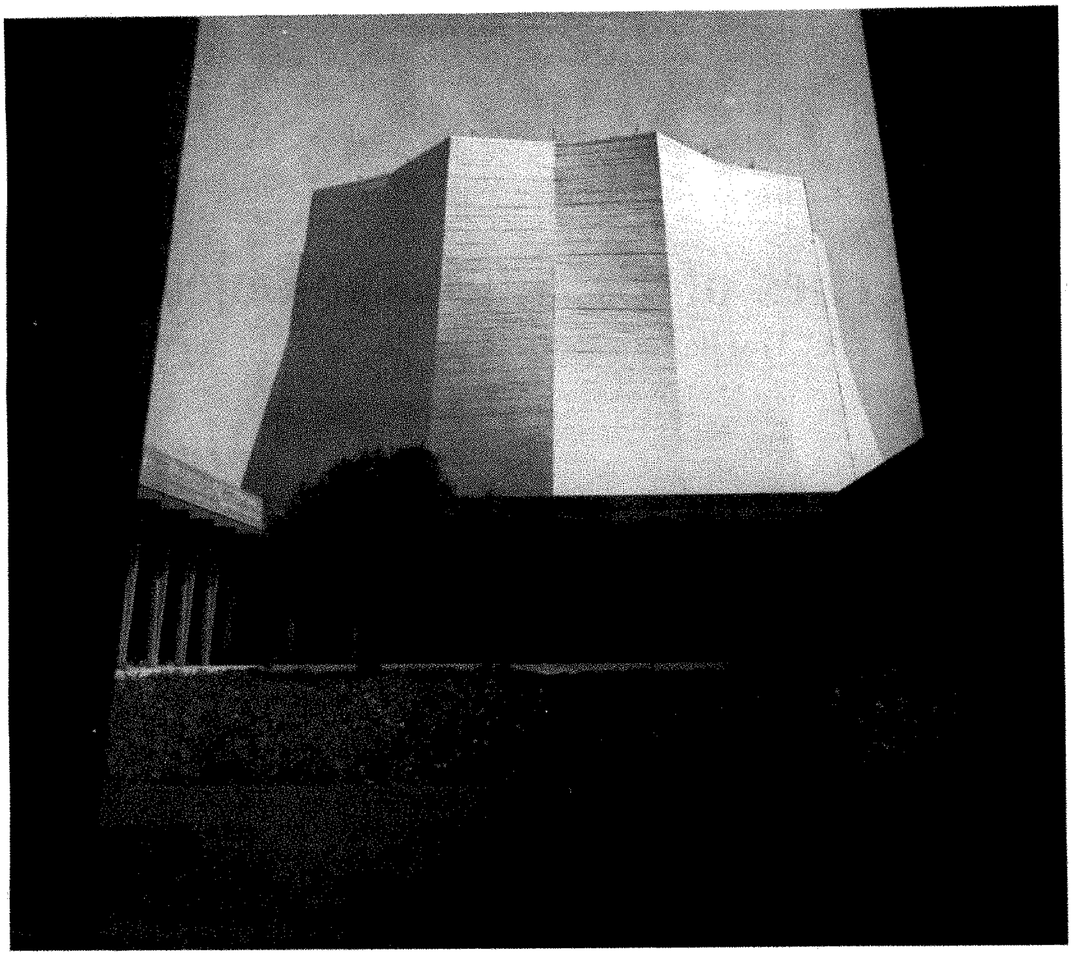

Fig. 2 Experimental nuclear reactor at Nahal Soreq.

main resource we have, then this is what we have to develop.

Such an approach should attempt to create the proper climate for investing in science-based and other sophisticated industries; it should encourage institutions of higher learning to launch courses with the aim of producing not just more academics and technicians, but the kind of professionals needed for the desired industries. While pushing to create innovation centres in the universities, the government should adjust its financial support and tax incentive systems to benefit the more desired sectors of the economy; and last but not least: it should drive to create a new image for the scientist and engineer who work in applied research and industries.

The agricultural sector could provide a good example how successful cooperation between scientists, engineers and educated farmers brought about the fantastic achievements of Israeli agriculture. This sector supplies the country today with most of her essential foods, under semi-arid conditions and a severe shortage of water. It enables the settling of new and virgin land and consolidates a more balanced economy, diminishing Israel's dependence on imports. Between 1950 and 1970, agricultural production in Israel increased sixfold although the numbers employed in agriculture had decreased. In many commodities (such as wheat, cotton, milk and vegetables) Israel has reached world records per unit of production. These achievements were made possible through the application of experimental results to field conditions, introduction and development of new species and varieties, adopting modern methods and techniques—above all a joint effort.

Immediately after the 1967 war, Israeli extension officers aided Arab farmers in the occupied territories to introduce modern methods of soil cultivation, water use and food production. These extension officers were merely a small group among hundreds of Israeli scientists, physicians and technicians who were engaged during the 1960s and the beginning of the 1970s in foreign aid, mostly to developing countries.

This agricultural model of developing new manpower through developing new occupations, to reap high quality achievements could serve industry and research and development services. Israel offers a particularly attractive climate for technologically intensive and research intensive industries. Not only do such industries enjoy high quality manpower, but they also have an advantage over most Western countries as salaries here are lower than in most industrial countries. Thus the cost of research and professional manpower is significantly reduced. Most science based industries in Israel are less than ten years old, but they already embrace a variety of fields and are developing fast. Another example of successful science-based industry is the military industry of Israel.

A well-defined central policy should aim at putting academic manpower at the top of the list of priorities; it should nurture such manpower in every avenue of life; it should create a general feeling of the importance of this manpower to the country and should attempt to provide every graduate student, immigrating scientist or returning scientist with an answer to the question—What kind of professional work could he do in Israel? This policy should also seek to develop the infrastructure and technology suitable to the small size of Israel and to her specific social and political problems.

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article

Radio properties of BL Lac type objects

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New radio observations of nine BL Lac type objects are discussed. It is shown that in their radio properties, these objects range from highly variable sources with a strong centimetre excess to non-variable sources with comparatively steep spectra. All the evidence strongly suggests that the most variable BL Lac type objects are extremely young.

SINCE 1970 we have been monitoring the flux and polarisation of 87 compact radio sources using the NRAO three element interferometer at $\lambda\lambda$ 11.1 and 3.7 cm. Among the sources observed regularly were several members of the class of objects similar to BL Lacertae[1,2]. The radio behaviour of the better known BL Lac type objects seems to be characterised by large amplitude outbursts on very short time scales, and they seem to be extreme examples of the variable radio source phenomenon. We discuss the results of our own monitoring programme and compare the radio properties of BL Lac type objects with those of other variable radio sources.

Strittmatter *et al.*[1] give the main characteristics of BL Lac type objects as: (1) rapid variations in flux density at optical, infrared and radio wavelengths; (2) a spectrum such that most of the energy is emitted at infrared wavelengths; (3) no lines visible in low dispersion optical spectra; (4) strong and rapidly varying polarisation at optical and radio wavelengths. Here we consider BL Lac type objects as a class of optical objects without being prejudiced by their radio properties. Characteristic (3) is an insufficient definition since it includes various galaxies and white dwarf stars. Here we define a BL Lac type object as one that exhibits strong and rapidly varying non-thermal radiation at optical wavelengths and has a very small line/non-thermal continuum ratio.

There are at least 20 of these objects now known. In this paper we restrict discussion to the 10 objects known before March 1974. Since information on these 10 sources is scattered throughout the literature, a summary of relevant references is given in Table 1. Five of them (BL Lac, OJ287, 0048−09, W Coma, AP Lib) have been extensively observed by us for some years, and four others (4C47.08, 0912+29, 1101+38, 1215+30) have recently been added to our list and have been observed briefly. (The ten newest objects[3-6] are now also being monitored regularly and will be discussed elsewhere.)

Spectra and flux variations

The radio spectra of the 10 sources are given in Fig. 1. The BL Lac type objects are not homogeneous in their radio properties. Although none shows steep spectra characteristic of extended optically thin sources, not all show the extreme centimetre excess of BL Lac and OJ287.

We find the usual correlation between centimetre excess and degree of variability[21]. Of the sources we have observed extensively, BL Lac, OJ287 and 0048−09 show rapid large amplitude variations. Stull and Carpenter[28] found no evidence of variability for W Coma. We find clear evidence of variability, however, in particular in the polarised flux density but with a smaller amplitude and longer time scale than for the first three sources. AP Lib shows at most marginal variations[31,32]. This is consistent with our own observations so far. Figure 2 shows our observations up to March 1974 of these five sources. The observing technique and detailed data will be given elsewhere.

Of the four sources added only recently to our programme, 4C47.08 has shown a large decrease in flux density at both λ 11.1 and λ 3.7 cm in only a few months and seems to be similar to the other sources with a strong centimetre excess. The sources 0912+29, 1101+38 and 1215+30 have shown no variations so far. These sources may be similar to AP Lb, although far more extensive monitoring is necessary to establish this firmly.

The remainder of this paper will be concerned with the four well studied variable sources (BL Lac, OJ287, 0048−09, and W Coma), and with a comparison of their radio properties with those of 21 other radio sources we have observed as extensively and which have also been found to be variable.

An unusual feature of the spectra of these four sources is that they have a positive spectral index over the whole range of wavelengths at which they have been observed. There is no sign of emission from optically thin regions that may be visible at long wavelengths. Such spectra are rare. In the compilation of 373 spectra by Veron, Veron and Witzel[51], there are only two clear cases and five marginal cases of sources which are optically thick at both 10 GHz and 100 MHz. Most of the well known variable sources, such as 3C279 and 3C454.3, seem to contain extended steep spectrum components which dominate the spectrum at long wavelengths. We shall return to this point later.

Table 1 Summary of references used

Source names	Optical properties	Infrared flux	Radio flux	Radio polarisation
0048−09 OB−080	7		8−10*	*
0300+47 4C47.08 OE 400	11	1, 12−15	11*	11*
0851+20 OJ287	1, 12, 16, 13, 17–20		10, 12, 16, 13, 21–23*	12*
0912+29	24		24, 25*	*
1101+38	26		26*	*
1215+30 ON 325	1, 27	14	25*	*
1219+28 W Coma ON 231	1, 27	14	28, 25*	*
1514−24 AP Lib	1, 29, 30	14	31, 32, 33*	*
1727+50 IZW−187	34, 35, 2		2*	*
2200+42 BL Lac	13, 1, 36–44	14, 13, 40, 45	14, 21, 46–50*	46, 49*

*, Our measurements.

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The four variable BL Lac type objects are among the most violently variable source in our programme in terms of both the amplitude and the time scale of the outbursts. We have attempted to assign a characteristic time scale to the larger variations for these and for the 21 other variable sources. The distribution of time scales is shown as a histogram in Fig. 3. It should be noted that this is necessarily a rather subjective procedure. Our observations were spaced at intervals of four to six weeks, so we cannot resolve time scales shorter than this. Most variable sources exhibit a range of time scales, and indeed OJ287 and BL Lac show small amplitude variations on a time scale of less than a day[12,13]. Also there are certainly objects other than BL Lac type objects which vary on a time scale of less than three months.

In Table 2, we compare the relative amplitude of the outbursts for the four BL Lac type objects and the 21 other variable sources. This is computed as $(S_{\max}-S_{\min})/S_{\text{average}}$, where S is the flux density. Median values of this quantity are given at both wavelengths for the two groups. These 21 radio sources include all those extragalactic radio sources known to be variable as of January 1972, and include such well known sources as 3C120 and 3C454.2. Again, although as a group the variable BL Lac type objects exhibit the most extreme behaviour, examples of other sources can be found which show just as large amplitude variations.

Linear polarisation

The linear polarisation of the four BL Lac type objects also shows important differences from the other sources. First, we note that for all sources changes in polarisation can be considerably more rapid and larger in amplitude than the changes in flux density.

It is often illuminating to represent a polarised flux density P with a position angle χ as a vector $P\exp(2j\chi)$ in the argand diagram. This is identical to plotting the Stokes parameters U against Q. Although the time behaviour of the polarisation becomes more difficult to follow, the advantage is that any constant contribution to the observed polarisation from non-variable components in the source is simply represented as a constant displacement in the diagram.

When this is done, a striking difference between the BL Lac type objects and the other sources is apparent. In Fig. 4 an example of each type of behaviour is shown.

The data for 0048−09 cover all four quadrants of the diagram. 1055+01 also shows large variations, but the data are well displaced from the origin and are contained in a cone subtending less than 90° from the origin. We have measured the angle of this "cone", $\Delta\varphi$, and the results are shown in Fig. 5.

It is interesting that the only source that shows variations in position angle at both wavelengths as large as those of the BL Lac type objects is 0607−15, which has been identified[52] simply as a "stellar object". It is also one of the more rapidly varying sources ($\tau\sim 5$ months), and it is possible that when optical spectra are taken, it may also turn out to be a BL Lac type object.

The centroid of the region covered by data points in these vector diagrams is an estimate of a constant or slowly changing component of the total polarisation; excursions from this centroid represent the polarisation of individual outbursts. The preceding results then indicate two things. First, for the BL Lac type objects at least, the polarisation of different outbursts has differing position angles, implying differing magnetic field orientations. Secondly, any constant contribution to the polarisation is quite small for the BL Lac type objects but comparatively large for the other sources.

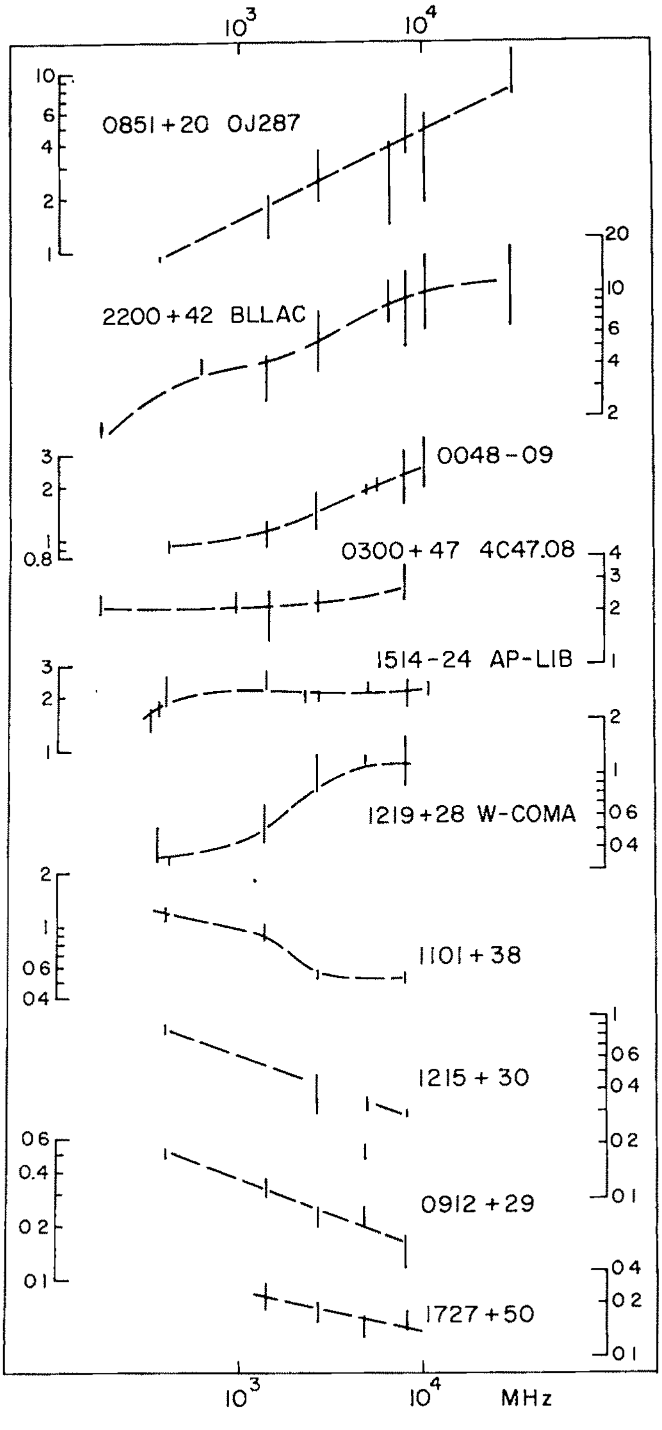
The effect is shown in another way in Fig. 6, in terms of the "average" polarisation at λ 11.1 cm. The "average" here is simply a vector average of all the observations for each source. Again, the average polarisation of the BL Lac type objects is small, although individual data points may have a much higher polarisation, and non-BL Lac type sources tend to have a much more strongly polarised constant component.

This result together with the large percentage amplitude variations and the absence of steep spectrum components visible at long wavelengths suggest that these four BL Lac type objects are extremely young objects which have yet to develop extended non-variable optically thin structures surrounding them. It is interesting that the only BL Lac type object known to have extended radio structure on the scale of seconds of arc is AP Lib (ref. 53), which does not show a strong centimetre excess, shows little evidence of variability, and has an average polarisation at λ11.1 cm of 5.4%.

Distances

There has been some controversy concerning the distances to the BL Lac type objects. Stein *et al.*[45] and Visnavathan[20] derived distances to BL Lac and OJ287 of less than 1 Mpc by using indirect arguments based on the radio and optical variabi-

Fig. 1 Radio spectra of ten BL Lac type objects. The data are taken from the references in Table 1. The error bars include the known range of variation. No effort was made to take into account differing absolute calibrations.



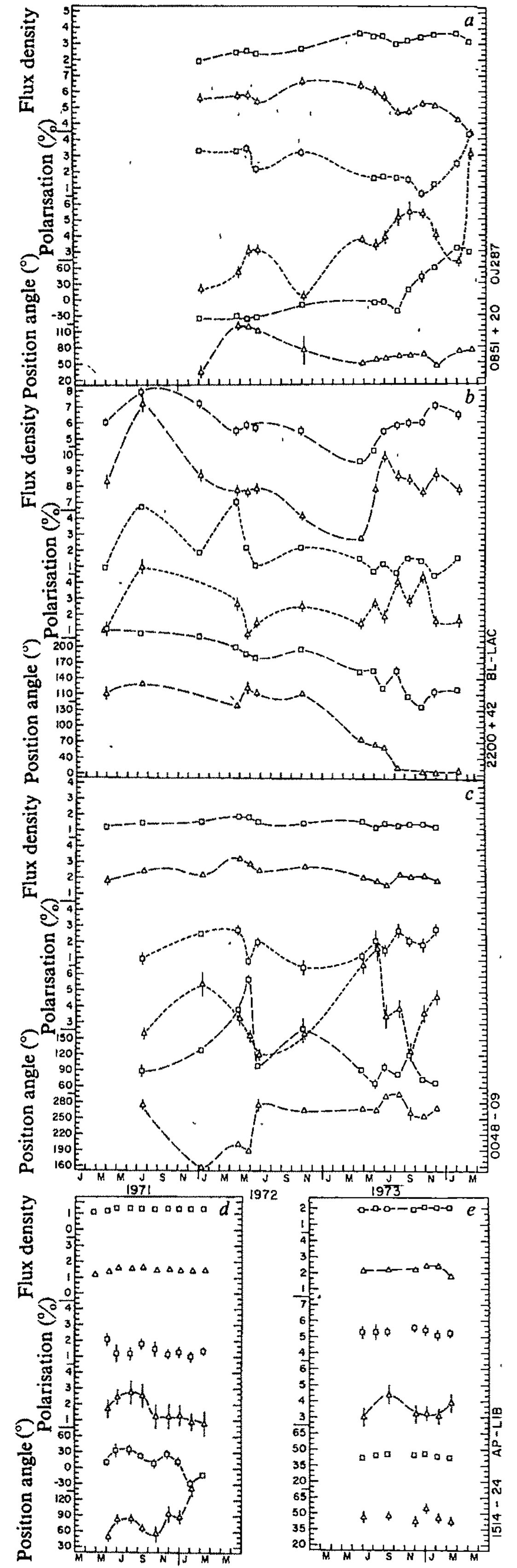


Fig. 2 The data for five BL Lac type objects The points represent the flux density (Jy), percentage linear polarisation, and position angle (degrees), at $\lambda = 11.1$ cm (□) and $\lambda = 3.7$ cm (△). The lines connecting the data points were drawn by hand simply as an aid to the eye.

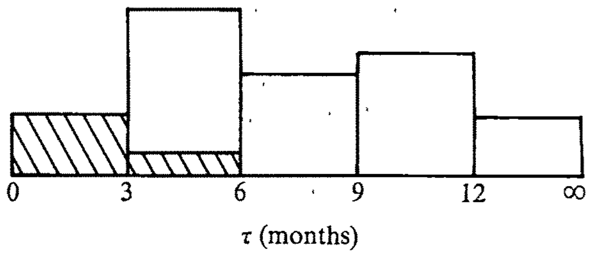


Fig. 3 Distribution of time scales for 25 variable sources. ■, BL Lac type.

lity data. But Oke and Gunn[36] have measured a redshift o 0.07 for the optical nebulosity surrounding BL Lac, implyin a distance of some 350 Mpc if the redshift is cosmological We shall discuss briefly the method used by Stein *et al.* an by Visnavathan, since similar arguments have also been use to show that some variable quasars must also be at very smal distances (see ref. 54).

The method is based on two physical arguments. First, th time scale of the variations should be less than the light trave time across the source ($r<c\tau$) where r is the radius of th emitting region. Secondly, if catastrophic inverse Compto losses are to be avoided, the energy density of the magneti field must exceed the energy density of the photons ($B^2/8\pi> 3L/4\pi r^2c$), where B is the magnetic field and L is the tota luminosity of the source. Combining these, an upper limit t the distance of the source can be written roughly as

$$D<6100\left[\frac{B}{1\text{ gauss}}\right]\left[\frac{c\tau}{1\text{ light yr}}\right]\left[\frac{\Delta s}{1\text{ Jy}}\right]^{-1/2}\left[\frac{\nu_c}{10^{11}\text{ Hz}}\right]^{-1/2}\text{Mpc}\quad(1$$

where Δs is the amplitude of the outburst, and ν_c is an uppe cutoff to the spectrum of the outburst.

If the source is expanding relativistically with a Lorent factor γ, then this limit may be increased by a factor $\gamma^{7/5}$ This is probably not very important compared with the othe uncertainties in evaluating equation (1).

Table 2 Amplitude of the flux variations

	BL Lac type	21 other sources
λ11.1 cm	55%	25%
λ 3.7 cm	70%	40%

There has been some confusion as to the appropriate value to insert in equation (1). We stress that these quantities refe only to the outburst, and not to the source as a whole. Also it is often implicitly assumed that the source is a unifor homogeneous region. There is considerable evidence agains this: (1) the optical and radio outbursts seem to be uncorrelated; (2) the polarisation at optical wavelengths is usuall very much higher than that at even the shortest radio wavelengths[12]; (3) the radio spectrum rarely shows a spectra index as steep as +2.5; (4) the uniform expanding clou model of Van der Laan[55] rarely fits the data[21]; (5) VLB observations reveal extremely complex structure on the smalles scales[56].

In view of this, it may be dangerous to derive magneti field strengths from the usual synchrotron self-absorptio argument. It is almost certainly wrong to apply a value of th field derived from radio data to the optical region, or a valu of the time scale taken from the optical variations to the radi region.

Sometimes the magnetic field strength in the optical regio

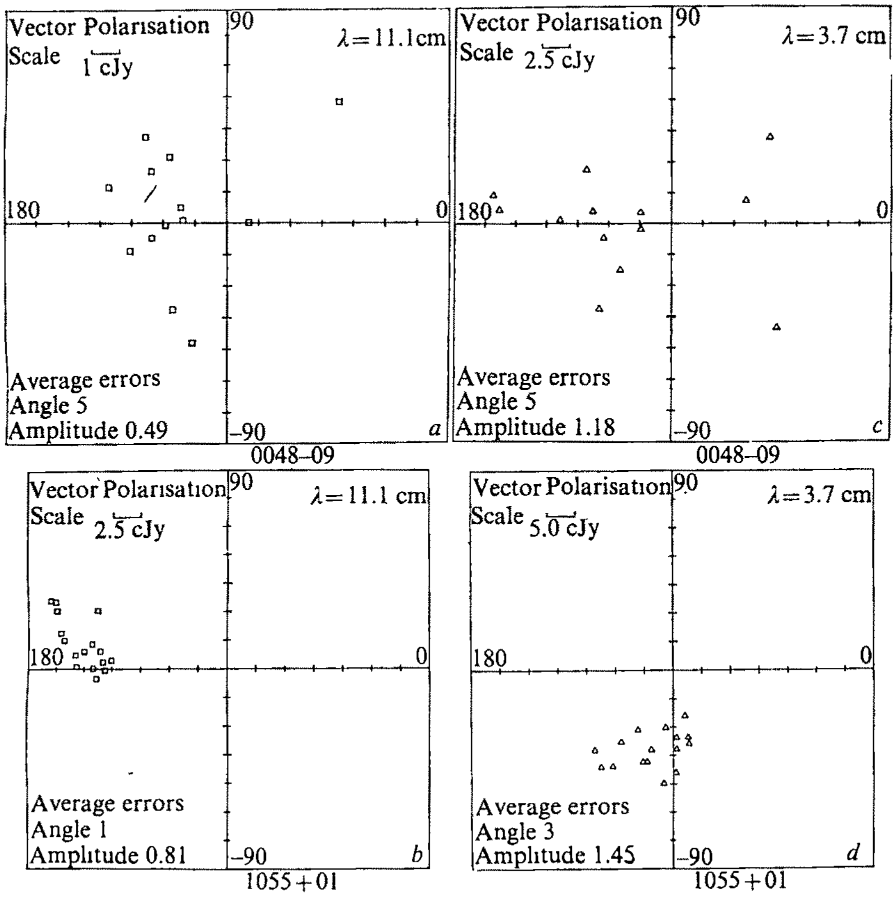


Fig. 4 The data for 0048–09 (BL Lac type) (*a*, *c*) and 1055+01 (non-BL Lac type) (*b*, *d*) plotted on the argand diagram.

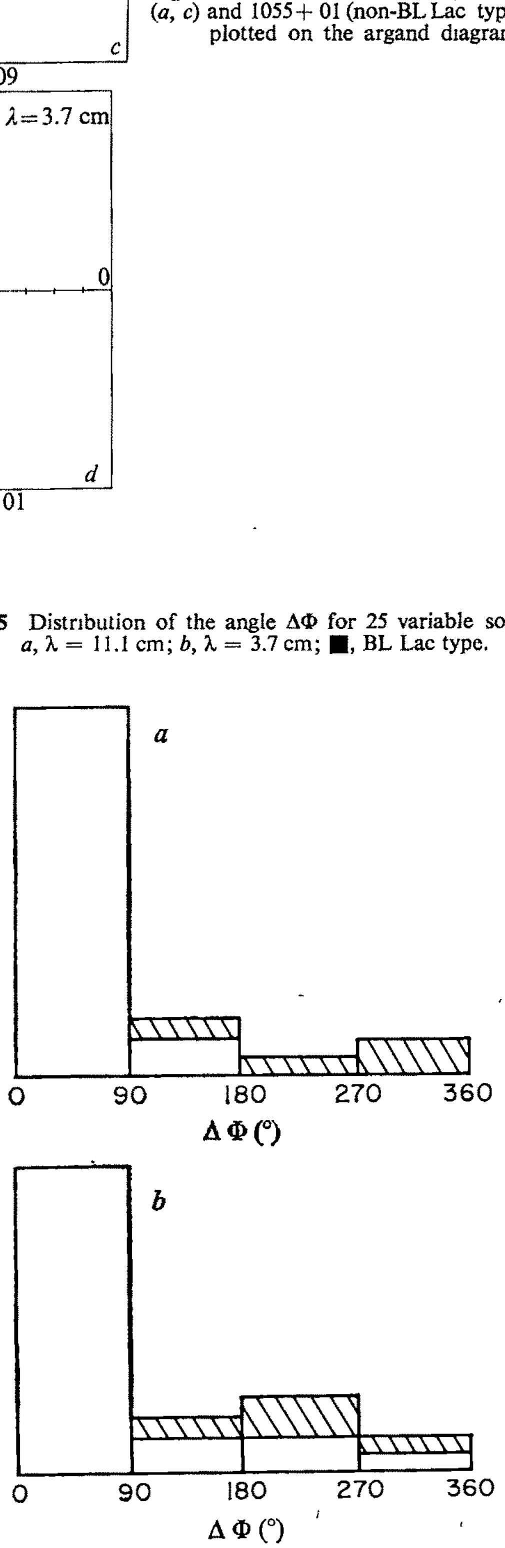
is estimated from the requirements that the half life of the electrons against synchrotron losses be greater than the time scale of the burst But Peterson and Dent[57] have given evidence that continuing injection of electrons takes place over comparatively long time scales in at least some radio outbursts. It is possible that the rapid fluctuations seen at optical wavelengths represent fluctuations in the injection rate for relativistic electrons rather than the development of an expanding cloud. Although this increases the energy requirements, we know so little about the source of the energy that this is hardly a strong objection.

If we insert typical values into equation (1), ($B\sim$0.1–10 gauss, $\tau\sim$0.1–0.3 yr, $\Delta s\sim$1–10 Jy, $\nu_c\sim 10^{11}$–10^{15} Hz), we obtain a range of distances from 200 kpc to 2,000 Mpc. In view of the uncertainties discussed above, and of the lack of a detailed physical understanding of these sources, we feel it is unwise to try and be much more specific. This estimate certainly allows the BL Lac type objects to be extragalactic objects at great distances, and is entirely consistent with the redshift of 0.07 found for the optical nebulosity surrounding BL Lac. Finally, a source with a diameter of 0.3 light yr at a distance of 100 to 1,000 Mpc would have an angular diameter in the range of 0.2×10^{3}–2×10^{-3} arc s. This is consistent with VLB observations[56,58]. Jones *et al*[59] have recently presented a more comprehensive account of the distance problem.

General nature of BL Lac type objects

If BL Lac type objects are defined solely by a very small optical line–continuum ratio, then as we have shown, they form a very nonhomogeneous group in their radio properties. In fact they show the whole range of properties exhibited by other compact extragalactic radio sources. Some of these objects have comparatively steep spectra and appear not to be variable, while others show a centimetre excess and are certainly highly variable. The three sources BL Lac, OJ287 and 0048–09 are among the most rapid and violently variable radio sources known, and seem to represent an extreme of the

Fig. 5 Distribution of the angle ΔΦ for 25 variable sources. *a*, λ = 11.1 cm; *b*, λ = 3.7 cm; ■, BL Lac type.



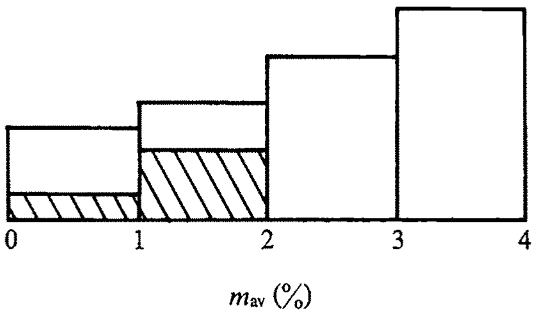


Fig. 6 Distribution of the mean polarisation at $\lambda = 11.1$ cm for 25 variable sources. $\lambda = 11.1$ cm; ■, BL Lac type.

variable radio source phenomenon. All the evidence is consistent with their being extragalactic objects at great distances. They may be among the most luminous objects in the Universe.

For the most variable BL Lac type objects, the spectrum, flux variations, and polarisation data all suggest strongly that they are extremely young sources which have not yet developed extended non-variable optically thin radio structure surrounding them. In this respect they may be pictured as "naked" radio sources.

This view is consistent with the results of Condon and Jauncey[60] who give evidence that BL Lac type objects may have been ejected recently at high velocity from nearby galaxies. It is perhaps tempting to label these sources as "streakers".

The National Radio Astronomy Observatory is operated by Associated Universities Inc. under contract with the National Science Foundation. This work was supported by the NSF.

Received March 3; accepted April 15, 1975.

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letters to nature

Radio brightness distribution of 3C273

THE radio source 3C273 was one of the first to be identified[1] with a QSO. The main features of the brightness distribution have been established from lunar occultation studies[2–5], which show that the source is double, with one component coincident with a 13 mag QSO or redshift 0.158. This component, 3C273B, is variable, and has an inverted radio spectrum. The component is compact, and details of its structure have been investigated with inter-continental (VLBI) interferometers[6–8]. The other component, 3C273A, has a normal radio spectrum and is identified with a faint optical jet lying in position angle 223° with respect to the QSO Component A has extended radio structure, and is not detected in VLBI observations[8]. The colours of the jet indicate that the optical emission, like the radio emission from component A, is non-thermal[9,10]. The optical jet of 3C273 is an extremely unusual feature, and it would be interesting to make a detailed comparison of the optical and radio structure associated with the component We provide here detailed information of the radio brightness distribution

The source has not been mapped by aperture synthesis techniques because of its proximity to the celestial equator (declination +2° 20′), which severely restricts the resolution available in declination for instruments with E–W baselines Lunar occultations of the source are of short duration, and the details of the brightness distribution obtained by this method are consequently limited by poor signal-to-noise ratio. In a previous study[11], we used interferometer observations to obtain strip brightness distributions along the major axis of 3C273 with a resolution of 7″, but were not able to resolve the structure perpendicular to this axis

We have now assembled observations of 3C273 made by ourselves and others using radio-linked interferometers based on Jodrell Bank. The outstation telescopes were situated at Wardle, Defford and Pocklington, which are respectively 23 km, 127 km and 132 km from Jodrell Bank. The observations were made at various wavelengths between 18 and

74 cm (frequencies 1,666–408 MHz), giving effectively eight different tracking interferometers with projected spacings up to about 150 kλ. The maximum resolution available is of the order 1.5″. A plot of the coverage of projected spacing (u, v) is shown in Fig. 1.

The double structure of 3C273 appears in the interferometer observations as a pattern of repeated maxima and minima in the fringe amplitude across the u–v plane. If components A and B were both unresolved, the loci of maxima and minima would be parallel straight lines. In fact, the observations show that the loci depart from this behaviour in a systematic way. Furthermore, the values of the maximum and minimum fringe amplitude also change systematically across the u–v plane. The data show that one component is unresolved at these spacings, and that the variations are entirely due to the resolution of the second component. The flux density of the unresolved component agrees with the flux density of component B derived from lunar occultation studies[12]. We therefore use component B as a phase reference source, and by noting the amplitude and exact position of each maximum and minimum, we have deduced the amplitude and phase contributed by component A alone.

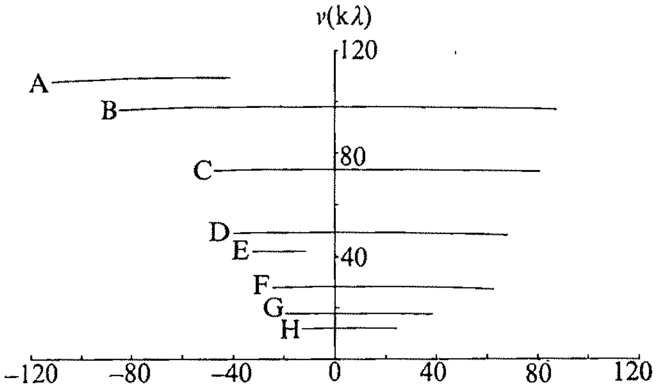

Fig. 1 The projected interferometer spacings on the resolution (u–v) plane. The observations A and B were made at λ74 cm using the Jodrell–Malvern and Wardle–Malvern interferometers respectively. Observations C are from the published data of Anderson and Donaldson at λ74 cm (ref. 15). The observations D to H were made with the Jodrell–Wardle interferometer at λ18, 21, 31, 49 and 74 cm respectively.

To combine the observations at different wavelengths, we have normalised the fringe amplitudes of component A by dividing them by the flux density of A obtained from lunar occultation data[12]. The values of normalised visibility vary smoothly across the u–v plane in a manner that depends solely on resolution and not on observing wavelength. We conclude that the structure of component A is independent of wavelength over the range 18–74 cm. This is consistent with our previous study at lower resolution[11].

We have performed a two-dimensional Fourier transform of the visibility data derived from all the baselines to produce a synthesis map of the brightness distribution of component A. The map has been treated by the CLEAN procedure[13] to reduce the unwanted sidelobe responses caused by the incomplete coverage of the u–v plane The resulting 'cleaned' map is shown in Fig. 2, together with a representation of the restoring beam, which has a half power width of 1.5″. A small region of negative brightness to the south of the main peak is a remnant of the main negative sidelobe produced by the original Fourier transformation, and indicates the confidence level of the map. Errors resulting from noise and confusion are much less than the lowest contour interval.

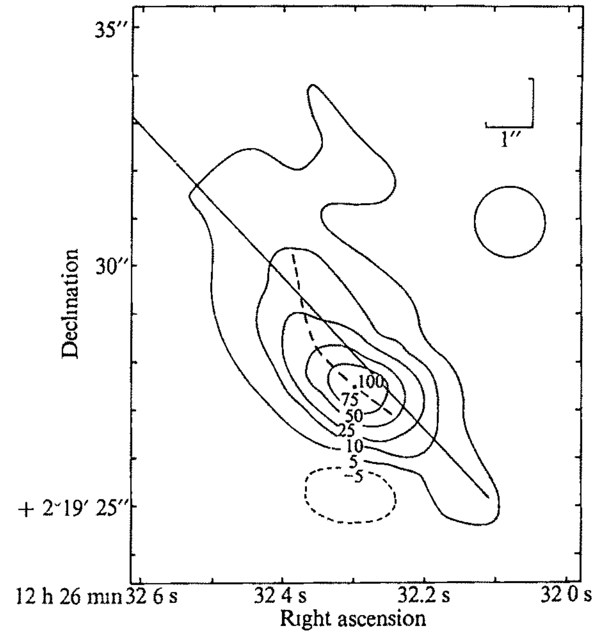

Fig. 2 The radio brightness distribution of 3C273A. The contour levels are expressed as percentages of the highest brightness. The ridge of peak radio brightness is represented by the curving dashed line. The solid line in p.a. 223° is a schematic representation of the optical jet (tip at lower right). Component B (the QSO) lies on the extrapolation of this line to the left, 25″ from the tip of the optical jet. Circle denotes beam used.

Schmidt[9] originally described the optical jet as a faint wisp which has a width of 1–2″ and extends away from the QSO in p.a. 223°. The optical brightness distribution along the jet has been measured photoelectrically[10]; the feature is first detected about 10″ from the QSO and fades into the noise at a distance of about 25″. There is no obvious peak in the optical brightness.

The radio brightness distribution of component A shows a pronounced peak of emission approximately 0.5″ south of the axis of the jet. The peak may contain unresolved structure. We believe that the uncertainty in the optical position is such that the radio peak could in fact coincide with the axis of the jet. There is an extended region of radio emission at the inner or North following end of component A. Although the surface brightness of the emission is low, the integrated flux from this region as a whole is appreciable. There may also be a low brightness extension to the outer, South preceding, tip of the component. The integrated flux density of this extension is low, however, and the reality of the outer feature is not firmly established.

We show the ridge line of maximum radio brightness as a dashed line in Fig. 2. The extended region at the inner end of component A is clearly displaced to the north of the nominal axis of the optical jet The angular displacement is 1–2″, which corresponds to a linear displacement of 3–7 kpc if 3C273 lies at the cosmological distance suggested by its redshift. The displacement would be even more marked if the radio peak actually lies on the axis of the optical jet. We emphasise that this skewness in the brightness distribution of component A is not produced as an artefact of our analysis procedure, but is a real feature which may be detected in the original interferometer data.

Harris[14] has noted a number of double radio sources in which the components show non-aligned structure. It seems that 3C273 can be included in this category. Harris discusses some possible interpretations of this phenomenon. In the case of 3C273, however, the alignment of the optical jet makes the problem of explaining the non-alignment of the radio component even more severe.

We thank J. F. C. Wardle and P. N. Wilkinson for providing details of their observations of 3C273 at λ49 and λ21 cm, and B. Anderson, R. J. Davis, J. A. Gilbert, P. Haves and H. P. Palmer for assistance with the remaining observations. R. Harten provided the Fourier transform and CLEAN programs with which Fig. 2 was produced.

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Received March 27; accepted April 16, 1975.

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Comparison of sunspot periods with planetary synodic period resonances

FOR decades, the closeness of the 11.1-yr cycle of solar activity and the 11.86-yr period of Jupiter has been noted, along with some speculation that the planets may have a causal effect on triggering solar activity. Two studies[1,2] have presented a planetary function which stays in phase for hundreds of years, and both of them considered the influence of the planets with large values of mass/(distance to Sun)^3 or (m_i/d_i^3), namely Jupiter, Venus, Earth, and Mercury, although Mercury effects were not evaluated quantitatively in either case because of the assumption that they would represent a short term perturbation. Okal and Anderson[3] indicate that Mercury effects can be important and that the magnitude of the tides of the Sun do not have the same power spectrum as the solar activity. Although this paper does not consider Mercury, it presents a more general model than just a tidal concept; tides are likely to be less important than torque effects on the oblate spinning Sun, for example. The study reported in ref. 3 was limited to an evaluation of the maximum tidal potential. Lack of correlation of this function with solar activity is not equivalent to reducing the credibility of all planetary hypotheses of solar activity.

Table 1 Dependence of planet synodic period beat period on synodic period variation. Top row and left column based on data from 1930–60. A 0.1% change in a synodic period changes the beat period by about 5%

	T_{EJ} = 1.0909 yr Low	T_{EJ} = 1.0921 yr Mean	T_{EJ} = 1.0932 yr High
T_{EV} = 1.5970 yr Low	22.14 yr	21.19 yr	20.40 yr
T_{EV} = 1.5987 yr Mean	23.16 yr	22.13 yr	21.26 yr
T_{EV} = 1.6007 yr High	24.49 yr	23.34 yr	22.38 yr

To determine the possible influence of several planets simultaneously on the Sun, it is convenient to consider them two at a time, as reflected by the synodic periods (periods between successive conjunctions). For zero eccentricity orbits, the (m_i/d_i^3) effects are exactly the same at conjunction as at opposition, and therefore one-half the synodic period is the repetitive reinforcement period. This is shown clearly in Fig. 1, which represents by a cosine function a forcing function of periods $T_{ij}/2$. In the figure, the ordinates are the cosine of twice the angle between planets i and j, and the function is shown at 0 yr, near 11 yr, and near 22 yr, assuming that at time $t = 0$, $\theta_E = \theta_V = \theta_J = 0$. Twice the angle is consistent with a (m_i/d_i^3) term physically associable either with tidal effects or with torque effects on the oblate spinning Sun. With all functions starting at a maximum, after 0.8 yr two are almost exactly out of phase and after 1.6 yr all are almost exactly in phase again, except for a time delay Δt. Considering now the EV and EJ curves, after enough (say n) 1.6-yr cycles, the accumulated Δt will be equal to one $T_{EJ}/2$ time interval. If we

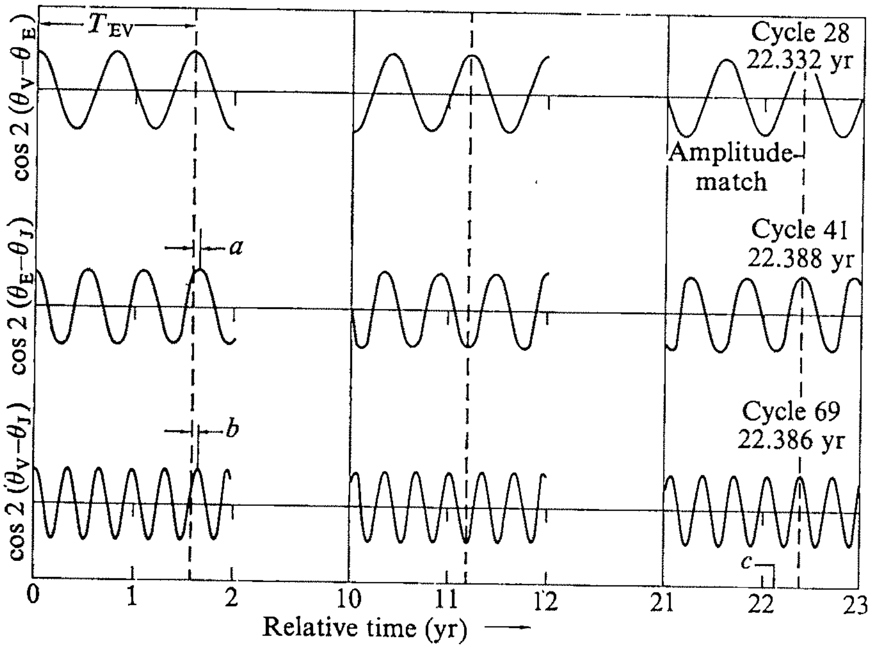

Fig. 1 Synodic angle cosine functions of planets for (m_i/d_i^3) effects: Earth–Venus (EV); Earth–Jupiter (EJ); and Venus–Jupiter (VJ), respectively. θ is measured with respect to an inertial reference such as the first point of Aries. Although the magnitudes match at 22.38+ yr, the nominal phase match occurs near 22.14 yr by equation 4. As seen from equation 6, this is conceptually equivalent to a simple harmonic of the difference frequencies of the EV and EJ curves. At 22.38+ yr in this zero-eccentricity model the planets and Sun would be lined up to within ±0.5°, but 139° from the starting line-up. At 22.14 yr, the planets, Sun, and original line-up at $t = 0$ are within ±48°, and at 11 07 yr, within ±24°. a, $\Delta t = \frac{3}{2}(T_{EJ}-T_{VE})$; b, $\Delta t = \frac{5}{2}(T_{VJ}-T_{VE})$; c, phase match, equation (4), 22.135 yr.

assume that the time required for this phase shift of one $T_{EJ}/2$ cycle to yield a synodic period resonance is equal to the period of the 22-yr solar cycle T_S, then for n T_{EV} periods

$$T_S = nT_{EV} \tag{1}$$

If the mismatch is $T_{EJ}/2$, then

$$n = (T_{EJ}/2\ \Delta t) \tag{2}$$

and

$$\Delta t = (3T_{EJ}/2) - T_{EV} \tag{3}$$

from Fig. 1. Since $T_{EV} = T_V T_E/(T_E - T_V)$ and $T_{EJ} = T_E T_J/(T_J - T_E)$ for circular orbits, then from equations (1), (2), and (3)

$$1/T_S = (3/T_V) - (5/T_E) + (2/T_J) \tag{4}$$

The same result is obtained using $n = (T_{VJ}/2)/\Delta t$ and $\Delta t = (5T_{VJ}/2) - T_{VE}$; or alternatively using $T_S = 3nT_{EJ}/2$, $n = T_{VJ}/3\ \Delta t$ and $\Delta t = (3T_{EJ}/2) - (5T_{VJ}/2)$, using the same logic to find resonances.

Using $T_V = 0.6152104$ yr
$T_E = 1.0000388$ yr
and $T_J = 11.862238$ yr in equation (4)

we obtain $T_S = 22.1353$ yr (5)
and $T_S/2 = 11.0676$ yr

Notice on Fig. 1 that at about 11 yr there would have also been an alignment of minima in the two lower curves, corresponding to a one-half cycle phase shift in contrast to a full cycle phase shift at about 22 yr.
Since expression (4) can also be written as

$$(1/T_S) = (3/T_{EV}) - (2/T_{EJ}) \tag{6}$$

the variation of the synodic periods due to eccentricity can have a large influence on the computed value of T_S. The mean synodic period over a large number of 11+ yr periods and its variability was determined by the examination of computer-generated conjunction–opposition data from 1930 to 1960.

$T_{EV} = 1.6007$ yr high
$= 1.5987$ yr mean (20 samples) (1.59872 yr theoretical, zero eccentricity)
$= 1.5970$ yr low
and
$T_{EJ} = 1.0932$ yr high
$= 1.0921$ yr mean (40 samples) (1.09211 yr theoretical, zero eccentricity)
$= 1.0909$ yr low

The influence of the variation of T_{EV} and T_{EJ} on T_S in equation 6 is shown in Table 1. A small change in planetary synodic period can lead to large changes in the value of T_S. For example, the nine values of Table 1 have a mean of 22.28 and a standard deviation of ± 1.2, to be compared with the mean and standard deviation of the real 22-yr cycle as follows:

$T_S = 22.35 \pm 1.76$ yr (minima, preceding odd cycles)
$T_S = 22.23 \pm 2.09$ yr (minima, preceding even cycles)
$T_S = 22.13 \pm 2.47$ yr (maxima, even cycles)
$T_S = 22.13 \pm 2.93$ yr (maxima, odd cycles)

Preceding sunspot data were taken from Waldmeier[4] using data from 1610.8 to 1957.9. The values of Table 1 are based on a 30-yr interval only instead of the 350-yr sunspot data base. Also, the values of T_{EJ} and T_{EV} are correlated because motion of the Earth at perihelion, for example, would have a tendency to reduce T_{EJ} and increase T_{EV}. This would have the effect of further increasing the expected resonance period variations from the zero eccentricity value of 22.135 yr, and can be inferred from Table 1.

This work shows not only a good correlation between planetary synodic period resonances with solar activity period, but also a rough correlation between the variations of the resonance period resulting from orbit eccentricity and the variations of the sunspot period. These correlations could be considered as a motivation for reassessing the physical mechanisms and attendant energy production magnitudes associated with all $(m_i/d_i{}^3)$ effects of the planets on the Sun in addition to the tidal effects considered in refs 2 and 3.

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Received February 3; accepted April 14, 1975.

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Radio wave propagation and ionisation of meteoric constituents

WE have studied from Calcutta (22°34′N; 88°24′E) the effect of meteoric ionisation on radio wave propagation characteristics in the low frequency band, where most radio aids for aerial and marine navigation operate. For the past few years we have been recording during the daytime the field strength of a radio navigational signal on 280 kHz (call sign JR) transmitted from Jessore (23°10′N; 89°10′E), about 110 km from Calcutta. Preliminary results indicated that meteors can produce significant ionisation below the ionospheric regions around 85 km responsible for the reflection of the low frequency signal, causing sizeable radio absorption events during major meteor shower activities[1,2]. Here we present new results obtained from a detailed analysis of the data for the periods of major meteor showers during a one year period from June 1972 to May 1973, which indicate that the dissipation of the ionised trail produced along the path of a meteor depends on the ionic characteristic of the trail.

The receiver used is basically a low noise superheterodyne receiver with bandwidth 2 kHz and gain 120 dB; the aerial used is of the inverted L type with vertical and horizontal parts 10 m and 50 m long, respectively. The effective height and the terminal capacitance of the aerial are respectively 9.17 m and 304 pF. The aerial is connected to the receiver input through a small capacitor (1 pF) to reduce the coupling of the antenna to the receiver. For a vertically polarised radio wave travelling horizontally towards the antenna, the vertical part of the inverted L-type antenna picks up the signal, while the horizontal part effectively improves the current distribution in the vertical part and effectively increases the signal pickup. If, however, the signal contains a horizontal component of polarisation, as may be expected for the sky wave, the horizontal part of the antenna will pick it up and add vectorially to the voltage picked up by the vertical part. The longer horizontal part of our inverted L antenna aligned in the direction of transmission picks up the horizontal component of the sky wave, which is larger than the vertical component because of the geometry of the ray path involved. Further, the antenna launching factors (F_T and F_R, T and R referring to the transmitter and receiver respectively) will in the case of poor ground conductivity reduce the ground-wave field significantly, while the sky wave component for the propagation distance involved is practically unaffected. In fact, the ground conductivity in the receiving location, which is situated in the heart of Calcutta, is extremely poor, being

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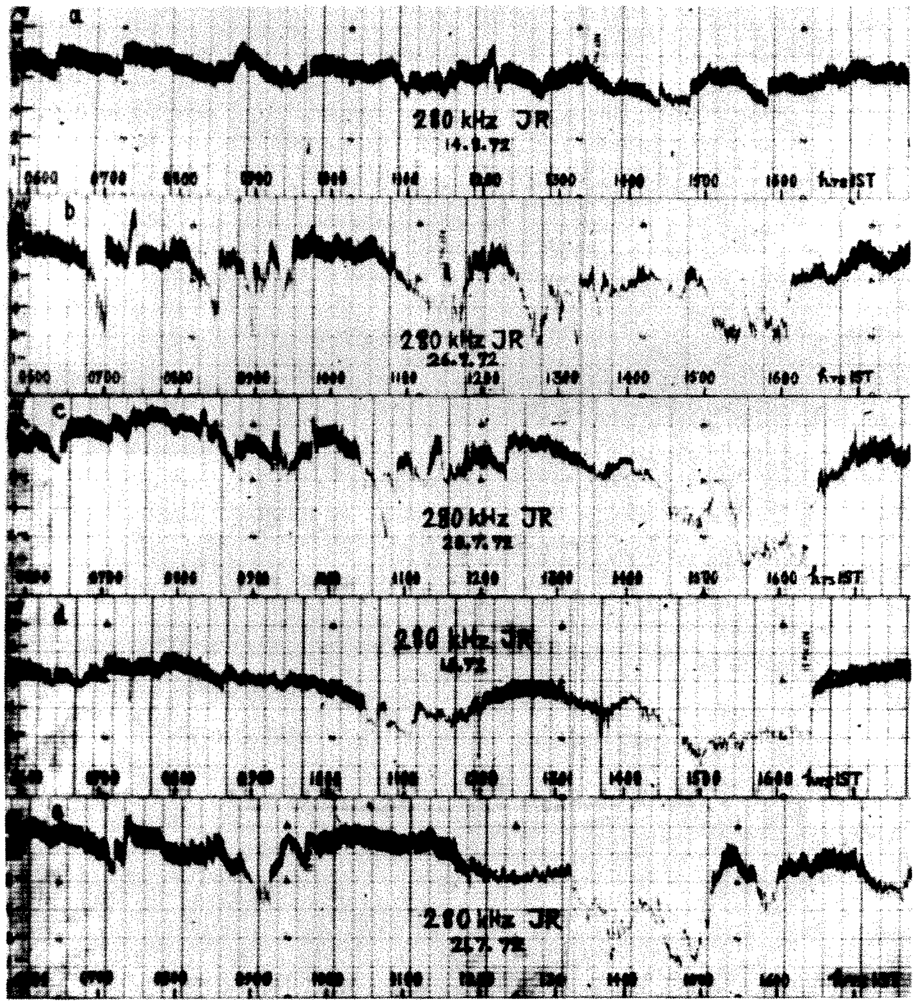


Fig. 1 Photograph of typical records showing the effect of meteor ionisation on the field strength of the low frequency signal. The ordinate shows the receiver input $(\mu V)^{1/2}$.

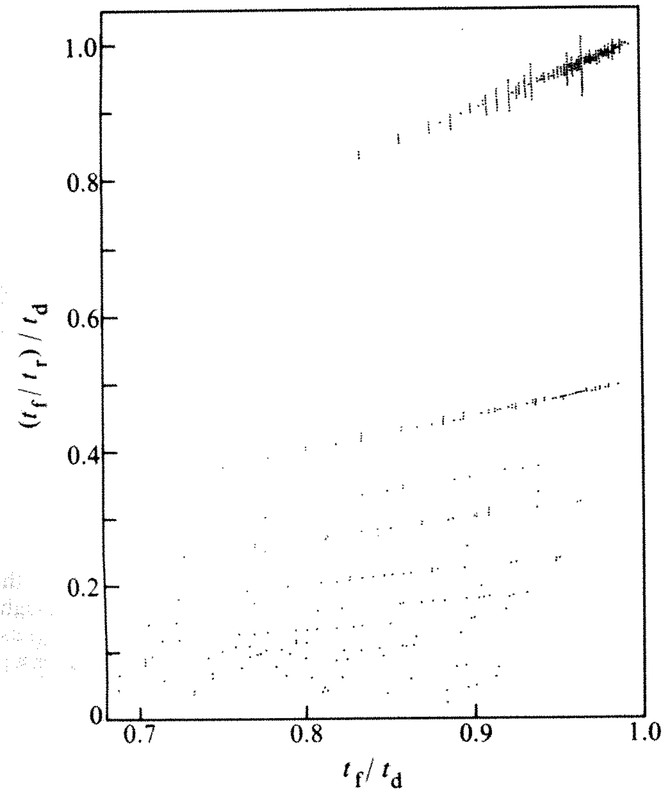
about 10^{-4}–10^{-5} times that of seawater. In addition since the transmission frequency is 280 kHz, which is much less than the gyrofrequency of 1.2 MHz involved, and the plane of propagation is inclined to the magnetic meridian at a large angle of about 45°, the presence of the Earth's magnetic field would reduce the absorption of the sky wave significantly[3]. The combined effect of these propagational factors make the sky wave–ground wave ratio significant so that the measured field shows up ionosphere changes.

The absorption process involved in the present case is predominantly collisional and the phase height remains fairly constant. Therefore, phase changes of sky wave at such times could be ignored.

Some of the typical records showing the effect of meteor ionisation on the field strength of the low frequency signal involved are reproduced in Fig. 1. Figure 1*a* shows a sawtooth type of fade out (SF) pattern in the form of a gradual fall of field strength starting successively at about 0600, 0623 and 0715 IST followed by sudden rises occurring at about 0620, 0714 and 0840. A similar effect is also noticeable in Fig. 1*b* *c* starting at about 0600 and 1005. Each of these effects was, however, accompanied by an opposite effect appearing in the form of a gradual rise followed by a sudden fall. The opposite effects started (Fig. 1*b* and *c*) at about 0715 and 1115. In addition Fig. 1*b*, *c*, *d*, *e* shows a remarkable trapezoidal fade out (TF) pattern starting at about 1410, 1400, 1405 and 1150. In all these cases, the fall is again gradual whereas the rise is fast with a significant time elapsing between fall and rise. The absorption events occurred both during day and night. The results of a detailed analysis of the daytime records of the SF effects observed during the periods of the meteor showers for the one year period under consideration are presented in Fig. 2. During this period there were 474 distinct SF effects, presumably associated with major meteor shower activities. Figure 2 shows the ratio of the time of gradual fall of field strength, t_f, to the time of sudden rise, t_r as plotted against the time of gradual fall, normalised by dividing both the parameters by the total duration, t_d. The total duration, t_d, is just $(t_f + t_r)$ for the SF effects and is of the order of 30 min; visual meteors usually last only for a few tens of seconds. The ionisation in meteor trails, which affects the LF propagation, lasts for a significantly longer time, resulting in the observed absorption event. From Fig. 2 it is clear that there is a tendency for data points to lie on several distinct straight lines with a remarkably low scatter up to at least the seventh line from the top. The slope of the lines seems to decrease gradually from the top to the bottom. The scatter of the data points increases below the seventh line and no clear linear relationship is observable for very low values of $(t_f/t_r)/t_d$. We attempted to explore the nature of variation of data points for the period of an individual meteor shower. For this, the data collected during the period of detectable dates of meteors for different showers for the 1-yr period were plotted separately. and it was found that even for a given shower period they seem to lie on the same distinct straight lines with different slopes.

The ionospheric regions responsible for the reflection of the l.f. signal involved are near the "meteor height". But the height of maximum meteor ionisation depends on velocity and mass of a meteor, being higher for greater velocities and lower masses. The ionisation produced by meteors of different masses and with different velocities would, therefore, affect the propagation of the signal in different ways. When the ionised trail is formed at the absorption level of the low frequency signal involved, the signal is absorbed. Whether the effect appears as SF or TF is determined by the electron line density in the trail and it is believed that the SFs and TFs are the same type of effect but that in TFs the ionisation in the absorption regions is much larger causing the signal to be drowned in the background of noise plus ground wave. It has been found that a TF effect would occur from an ionised meteor trail having an electron line density one order of magnitude greater than that in a trail causing a typical SF absorption event. The opposite type of SF effect is likely to be produced by a similar meteor trail ionisation at the low frequency reflection level involved. The duration of the absorption events is governed by the lifetime of the meteor trail ionisation, which, in turn, depends on the way the trail dissipates. There are several factors such as diffusion, recombination, attachment, turbulence and wind shears which contribute to the dissipation of the ionised trail. Of these, ambipolar diffusion is the most important factor for the eventual dissipation of the meteor trail ionisation. By diffusion the ionised trail expands at a relatively low rate producing a radial distribution of electrons which assumes a Gaussian distribution. If r_c is the radius of the expanded trail when the volume density in the trail is N_c, the critical volume density for the wavelength involved, then the time variation of the absorption of the signal would be expected to follow the variation of r_c with time. From a knowledge of the critical radius r_c the duration of the absorption event could be estimated, and it has been found that it depends markedly on λ, the wavelength involved. Hines and Forsyth[4] show that the echo duration of v.h.f. scattered signal is proportional to the square of the wavelength. Since the wavelength involved in the present investigation is more than two orders of magnitude compared to that in a typical meteor radar observation in the v.h.f. band, the duration of the low frequency absorption event would be much longer. The duration also depends on the diffusion coefficient which, in turn, depends on height as well as the constituents of a meteor. Fourteen different materials are common in meteors[5]. Of these, the atoms and ions that have been found in meteor spectra[6] are: Al; Ca; Ca$^+$; Co; Cr, Fe, Fe$^+$, Mg, Mg$^+$, Mn, Na, Ni, Si, Si$^+$, Sr$^+$. The relative abundances of different materials in a meteor may again vary from meteor to meteor. A wide variety of meteor spectra are observed in the optical and infrared ranges and Millman's[7] classification of such a spectra indicates that the spectral class depends not only on the velocity of a meteor but also on the type of a particular meteor. Thus although the shower meteors with velocities in the range 30–50 kms^{-1} are usually of type X, there are a few of type Y. Again, shower meteors with velocities from 20–30 km s^{-1} may be of type X or Z. Even if the meteor compositions did not vary, the differences in velocities of individual meteor would alter the relative abundances of the ions available during ablation[8]. The distinctive abundances of ions in the trail, whether produced by the variation of meteoride composition or by differences in velocities of individual meteors would eventually control the diffusion rate in distinctive ways. It seems that the various lines shown in Fig. 2 may result from the meteor producing each ionised trail having a different ion as the most abundant constituent, because the ratio $(t_f/t_r)/t_d$ for a given value of t_f/t_d would depend on the diffusion coefficient D, which, in turn depends on the relative abundances of ions present in the trail. The uppermost line of Fig. 2 can be associated with the most abundant ion Fe$^+$, which, being a heavy ion, would result in a high value of $(t_f/t_r)/t_d$, while the second, third, fourth and fifth lines from the top may be associated with Mg$^+$, Ca$^+$, Ni$^+$ and Si$^+$ as the most abundant ions respectively. The scatter of data points lying below the seventh line of Fig. 2 may result from other less abundant ions in the meteor trail. Besides these, the progressively lesser number of data points for lower lines is indicative of a smaller abundances of the constituent ions responsible for a particular line. We note that observations[9] on the composition of meteors producing E_s ionisations in the night time ionosphere indicate that Fe$^+$, Mg$^+$, Ca$^+$ and Ni$^+$ are the main constituents of E_s ionisation, with decreasing orders of abundances. Further, the fact that the data points collected for the periods of different showers seem to lie on the same distinct straight lines indicates that the lines obtained result from the characteristics of the meteors, irrespective of the showers to which they belong.

Fig. 2 Scatter plot of the normalised ratios of the time of fall to that of rise of the SF effects against the normalised fall time.



The positive ions produced along the path of a meteor do not play any direct role in the ionospheric reflection of the radio signal but the relative abundances of the ions present in the trail controls the ambipolar diffusion of the trail, which is presumed to be the main cause of dissipation of the trail during absorption events.

The influence of meteor showers on low frequency propagation characteristics was also studied by Chilliers[10] who observed that the shapes of the curves showing the hourly average speed of fading for three low frequency radio waves on 70 kHz, 113 kHz and 200 kHz were very similar. He compared the mean fading speed on 70 kHz with the mean number of meteors observed by radio methods and found a close association between the speed of the fast fading and the rate of incidence of meteors on the ionosphere. Earlier work by Weeks and Stuarts[11], on the measurement of the phase of waves near 100 kHz for short propagation path, showed that the diurnal phase curve was non-symmetrical, the morning decrease in phase height often exceeding the sunset increase. Chilliers thought this non-symmetry in phase height to be caused by discontinuous nocturnal changes in the height of reflection and attributed the phenomena to meteors. Some evidence of the effect of meteor showers on v.l.f. propagation characteristics came from the work of Chilton[12] who observed that the night-time phase height of 16 kHz transmissions for Rugby–Boulder propagation path remained unaltered during the period of meteor showers. Whenever the shower had passed or the entire path had become sunlit, the phase lag decreased rather abruptly to the normal daytime value, while on other days it decreased in a regular manner during sunrise.

The study of the low frequency radio absorption events during meteor showers, if supplemented by observation on v.h.f. forward scatter signals from the region of the lower ionosphere at the low frequency path mid-point may prove to be useful in revealing the true relationship between v.h.f. propagation and low frequency absorption resulting from meteor trail ionisation and may provide us with a deeper insight into the physical process involved.

We thank Professors J. N. Bhar, M. K. Das Gupta for comments. One of us (B.S.) thanks the Council of Scientific and Industrial Research, India for financial assistance.

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Received November 4, 1975; accepted April 16, 1975.

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Laser depolarisation 'bright band' from melting snowflakes

LINEAR depolarisation ratios (δ) obtained with a continuous wave (CW) laser system demonstrate that melting snowflakes backscatter unexpectedly large amounts of depolarised energy. δ values derived from single-particle scattering interactions increase from ~0.5 (dry snowflake average) to ~0.7 for moderately melted snowflakes, providing evidence for the existence of a laser radar depolarisation 'bright band' analogous to that observed at microwave radar frequencies.

The ability to distinguish between spherical and aspherical scattering elements through remote sensing techniques is a consequence of the Mie theory which predicts that spherically symmetrical particles backscatter energy that retains the incident state of polarisation, whereas asymmetrical particles introduce a depolarised component into the returned energy. This principle has been used by microwave radar investigators for the discrimination of large raindrops and hailstones in convective storm clouds[1], and for the differentiation of water and ice particle clouds with laser radar (lidar)[2]. Melting snowflakes have also been observed to produce microwave depolarisation[3]; regions containing precipitation a few hundred metres below the freezing level are often marked by a highly depolarised, intensely reflective layer (the bright band). This region contains large aggregates of ice crystals which have developed a liquid water covering (greatly enhancing the radar reflectivity compared with that of dry snowflakes), but which still fall at speeds slower than the more symmetrical, severely melted particles below. Because of differences in the modes of interaction of energies at the microwave and optical frequencies, it is not clear that a bright band analogy would be observed with lidar. If present, however, much useful information could be gained from studies of this feature (as demonstrated by the many related radar findings), while the characterisation of the return from melting precipitation could lead to a lessening of the confusion presently found in the lidar polarisation measurements reported from clouds.

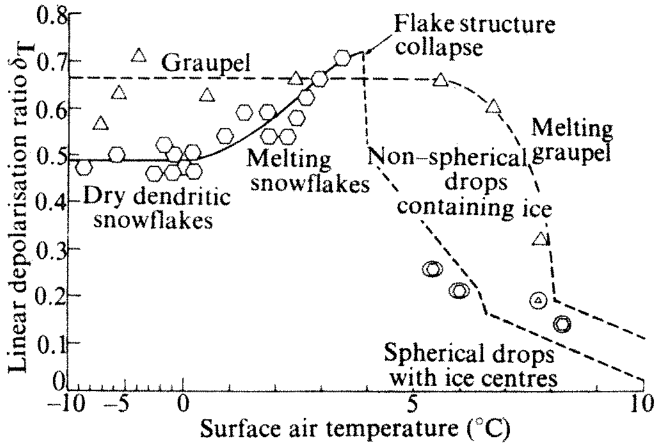

Fig. 1 Increase in depolarisation produced by melting snowflakes (hexagonal symbols) with increasing surface air temperature demonstrating the optical wavelength bright band analogy. Dashed lines represent the hypothesised backscattering behaviour of severely melted snowflakes and graupel particles (△) based on likely particle structural changes and geometric optics theory considerations, with data for non-spherical and spherical raindrops containing ice also shown.

An apparatus employing a vertically polarised CW helium–neon laser as a source has been designed to yield measurements applicable to those obtained by lidar to observe snowflake melting. Energy scattered in the backward direction from each particle falling through a small portion of the laser beam is collected by a receiver with a Glan-air polarising prism and dual photodetectors dividing the return into two components; the energy contained in the same plane of polarisation as that of the source, and that in the cross-polarised plane. The ratio of the orthogonal to parallel intensity components (δ, the linear depolarisation ratio) is measured by recording on film the scattering events displayed on an oscilloscope trace. Details of the experimental apparatus are given elsewhere[4].

Spring and autumn precipitation occurrences have been sampled at temperatures above and below freezing at Laramie, Wyoming. The results are shown in Fig. 1. The liquid water content and amount of loss of crystal structure displayed by melting ice particles can be estimated by surface air temperature, but as several factors may influence the rate at which melting occurs, some dispersion of the data with this temperature value can be expected. The 'total' linear depolarisation ratio, δ_T, has been used to denote the sum of all orthogonal component intensities over the sum of the parallel component intensities obtained from all scatterers observed during each interval, ranging from 112 to 823 (snowflakes) in number. This ratio approximates what would be observed by a polarisation diversity lidar interrogating an equivalent population of scatterers in a single pulse. But, it should be noted that actual lidar data can reflect multiple scattering influences (from scatterings among particles) absent in these measurements which cause an increase in the backscattered depolarised component. The magnitude of this effect on the return from ice particles is still not clear, but will probably be small if lidar design characteristics are used which limit the viewing of multiply scattered radiation[5].

From Fig. 1, dry snowflakes composed of large aggregates of dendritic ice crystals (hexagonal symbols below 0 °C) can be seen to produce δ_T values near 0.5, the same value as that displayed by populations of small artificial ice crystals[4]. Unrimed and lightly rimed planar and spatial crystals were sampled, with some indication that rimed crystal aggregates produced slightly greater δ_T values. Starting at temperatures slightly above 0 °C, δ_T for melting snowflakes increase to as high as 0.7, demonstrating the presence of an optical wavelength depolarisation bright band analogy. The energy returned from an individual snowflake consists of contributions from ice crystal specular reflections (which retain the polarisation of the source), multiple internal scatterings within a single crystal element, and inter-crystal scatterings. The latter events produce depolarisation through the rotation of the electric field vector of the incident wave. With snowflake melting, three structural changes take place which are believed to induce the increase in depolarisation; ice crystal specular reflecting areas are eroded, compaction of the crystal elements occurs, and a liquid water covering appears on the crystal faces. The alterations have the effect of increasing the highly depolarising inter-crystal scatterings, while subduing specular reflections. The presence of the additional water–ice interface may be particularly effective in increasing the probability of higher order reflections. The manner in which the effects of melting alter the frequency distribution of δ values from individual snowflakes of similar composition is illustrated in Fig 2. Two scattered intensity ranges are shown with the differences between the upper and shaded bars representing, for the most part, the sampling of single crystals or small crystal clusters. A typical dry snow case is shown at the bottom of Fig. 2; a rather narrow δ spectrum peaked near 0.45 with several $\delta \sim 0$ events from scatterings dominated by relatively intense specular reflections from one or more propitiously oriented crystal elements in the snowflakes. With melting, the δ spectra broaden and peak toward higher values. In addition, no $\delta \sim 0$ interactions of large intensity are present in the middle and upper spectra, although some events of small intensity occur. These are thought to result from slow falling crystals or clusters which had completely melted into small, spherical raindrops.

Increases in depolarisation are likely to proceed with melting until the hypothesised collapse of snowflake structure shown in Fig. 1 occurs, producing a rapid δ decrease resulting from the change in geometric cross section. The decline in δ_T from about 4 °C to 6 °C has been proposed to reflect the decreasing asymmetry and ice content of the drops, although only a few data points (depicted by circular and elliptical symbols) have been obtained in this region. Depolarisation is produced primarily as a result of scatterings with the enclosed ice elements. When a sufficient amount of ice has been converted to the liquid phase, the drop will assume a spherical symmetry but lack optical homogeneity. δ_T values for these particles will be low, since a large portion of the light backscattered by spherical particles results from front surface axial reflections and surface waves (responsible for the glory phenomena) which retain the polarisation of the source. A gradual decline in δ_T is thought to occur as the remaining ice content of the drops slowly changes phase. If the resulting raindrops are larger than a few millimetres in diameter, small amounts of depolarised energy may be produced because of the slight distortion in the sphericity of even these mono-phase particles.

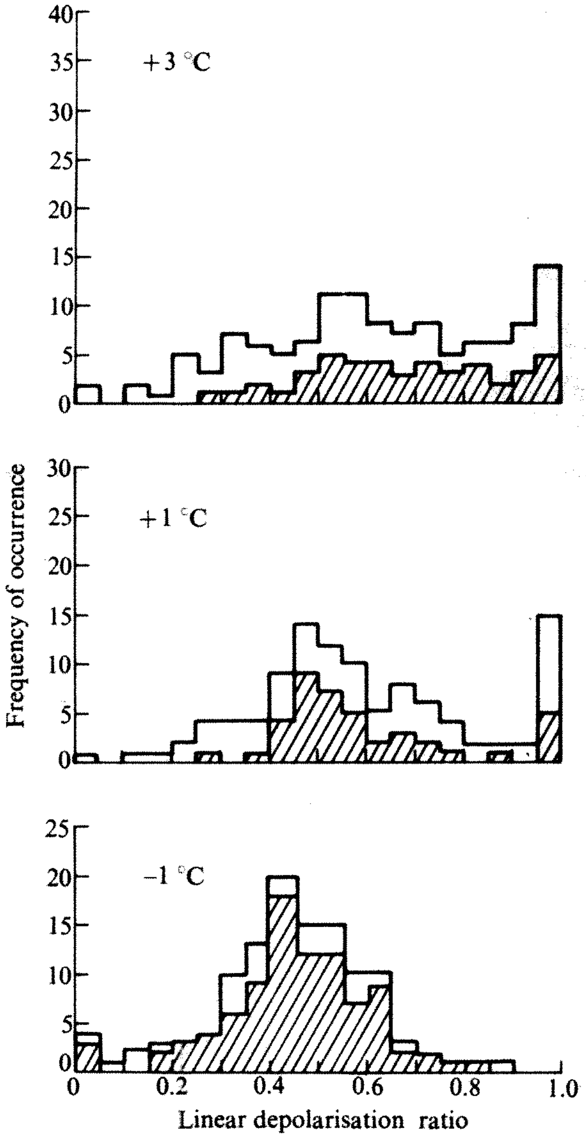

Fig. 2 Histograms of frequency of occurrence of δ values from individual snowflakes obtained from a continuous snowfall situation during which temperatures gradually decreased to below freezing. Changes in behaviour of δ spectra with snowflake melting (from bottom to top) aid in the evaluation of the causes of the depolarisation bright band analogy. Open columns, total scattering interactions; shaded columns, large intensity interactions.

Figure 1 also shows data from sampling showers of graupel particles (near spherical, opaque ice particles comprised chiefly of frozen cloud droplets collected during the elements' fall through a dense cloud). The difference in values between melting graupel (triangles) and snowflakes at temperatures >4 °C reflects partly the difference in fall velocities of the two species (since graupel fall at much greater speeds), but also because melting graupel display no fragile extensions, they gradually acquire a thickening liquid covering in contrast to the abrupt snowflake structural transition. A rapid decline in δ_T is suggested from the melting graupel data >6 °C. Although only a few data points are available, uniformly high values are indicated through much of the melting region which do not differ from those obtained at subfreezing temperatures. It is likely that a significant depolarisation bright band will be lacking for melting graupel, but as these particles display a return signature of $\delta_T \sim 0.65$ below 0 °C characteristic of their species, graupel and probably hail shafts as well may be discriminated within or below convective clouds with lidar techniques in suitable conditions.

Although the production of depolarised energy at microwave and optical frequencies is accomplished by vastly different scattering mechanisms, these measurements demonstrate the presence of a lidar depolarisation bright band analogy, and existing lidar data should be re-examined in the hope of identifying this previously unrecognised phenomenon.

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Received January 7; accepted April 14, 1975.

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Palaeomagnetic poles and a Proterozoic supercontinent

PALAEOMAGNETIC evidence does not support the model of a supercontinent made up of Laurentia and West Gondwanaland existing between 2,200 and 1,800 Myr BP. The palaeomagnetic poles (except those based on fewer than 10 samples) from the Laurentian Shield for the interval 2,200 to 1,800 Myr BP are shown in Fig. 1. The poles form a reasonably coherent array stretching from what is nowadays the Mackenzie delta to Peru. This polar path is referred to as polar track 5 (ref. 1), and the sense of apparent polar motion is probably from north to south.

The evidence for this is that the Dubawnt Group (pole D) Kahocella Formation (K), and the Martin Formation (MN) have ages between 1,800 and 1,900 Myr, and are younger than the Indin dikes (IN), which in their turn are younger than the Otto Stock (OS) and the Big Spruce Complex (BS). The age control of the results from the Mugford basalt, Wind River dikes, and the Otish gabbro (poles M, W, and OT) is not so good, but ages of about 2,000 Myr are the most probable. The errors in the results are such that track 5 is not defined as a single line but as a broad zone 15–20° wide. The 15° zone shown in Fig. 1 may correspond approximately to the standard error of the mean ($P = 0.67$) position.

The reconstruction of Fig. 1 indicates that about 2,200 Myr ago the Laurentian Shield was at a higher latitude, and this is consistent with the presence of glaciations in the Huronian. The latitude then decreased, and at about 1,800 Myr BP, the latitude ranges from 20–30° over the northern part of Laurentia, which is consistent with the presence of abundant red-beds and carbonates in younger Aphebian strata of the Canadian Shield. This broad correspondence between palaeoclimatic evidence and palaeolatitude indicates that in the interval 2,200–1,800 Myr BP the geomagnetic field was predominantly axial and dipolar. The change in latitude required by track 5 (that is, the minimum movement required) is about 100° at an average rate of about $3\ \mathrm{cm\ yr^{-1}}$.

One apparent inconsistency in this scheme is the position of the commonly accepted pole for the Nipissing diabase (poles N2, N4, and N6). The Nipissing diabase and the Otto Stock have ages that are not significantly different yet their apparent pole positions are almost 90° apart. Studies of the metamorphic aureole of the Otto Stock have shown that the remanence was acquired during cooling following intrusion[18]. But the time at which the Nipissing diabase was magnetised is not known, because a successful contact test and thermal demagnetisation studies have not been described. Much of the diabase has been regionally metamorphosed to low greenschist facies[24], and the iron minerals are commonly altered[16]. It is possible, therefore, that the remanence postdates the age of emplacement. Moreover the magnetisations observed in Nipissing diabase are complex, and directions other than those corresponding to poles N2, N4, and N6 have been observed (poles N3 and N5). Also the Abitibi dikes have one dominant (corresponding to pole AB1) and one subordinate (AB2) magnetisation, although the ages of the rocks from which they were derived are not significantly different[3]. Poles AB1 and N5 are close together, as are poles AB2, N2, N4, and N6. N3 falls between the two groups. Thus the magnetisations of the Abitibi and Nipissing diabases may be a complex mixture of as yet unresolved primary and overprinted magnetisations. The positions of the Abitibi and Nipissing diabase poles available at present may be regarded as uncertain, and all that can be said is that they probably lie near track 5.

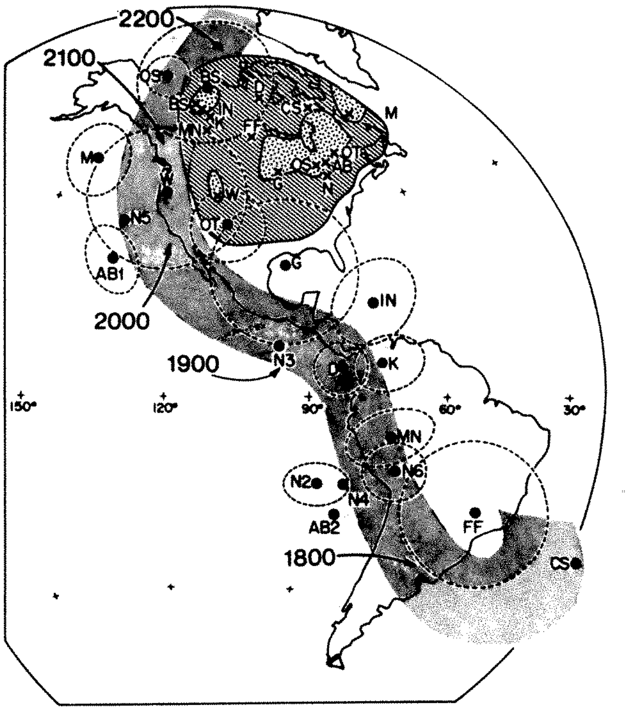

Fig. 1 Track 5. Pole positions for the Laurentian Shield for the interval 2,200 to 1,800 Myr BP. Error circles ($P = 0.05$) are given where these can be drawn with some confidence. The Archaean (stippled) and Proterozoic (oblique shading) terrains of Laurentia are distinguished. The poles and the localities from which they were obtained are indexed as follows: AB1 and AB2, Abitibi dikes[2], Rb–Sr isochron 2,147±68 Myr (ref. 3); D, Dubawnt Group, Rb–Sr isochron 1,830±26 Myr (ref. 4); G, Gunflint Formation[5], ≃2,000 Myr (ref. 6); IN Indian dikes[7], Rb–Sr isochron 2,093±86 Myr (ref. 3); K, Kahocella Formation, Rb–Sr isochron from associated volcanics 1,873±13 Myr (ref. 8); M, Mugford basalt[9], preliminary Rb–Sr isochron ≃2,300 Myr (ref. 10); MN, Martin Formation, K–Ar on associated dikes 1,835 Myr (ref. 11). Nipissing diabase: Rb–Sr isochrons[12,13] 2,160±60 and 2,150±50 Myr; five pole determinations N2[14], N3[15], N4 (selected sited from ref. 14), N5[14], N6[16]. OT Otish gabbro[17], age from indirect evidence ≃2,000 Myr; OS, Otto Stock[18], Rb–Sr isochron[19] 2,160±80 Myr[19]; W, Wind River dikes[20], maximum K–Ar age 2,060 Myr; CS, Volcanics of Cape Smith Belt[21] age uncertain but in the range 1,800 to 2,000 Myr, the lower limit being the probable age of metamorphism (the preferred age of magnetisation); BS, Big Spruce Complex[22], Rb–Sr isochron 2,170±40 Myr (ref. 23); FF, Flin Flon intrusions and metamorphic rocks[32] Rb–Sr isochrons 1,775±89 and 1,805±14 Myr.

The poles of track 5 are derived from many different parts of the Laurentian Shield, including the four Archaean blocks marked on Fig. 1 (the Superior Province—the largest, the Slave Province (marked BS), the Bear-tooth uplift (W), and the Labrador coastal section of the North Atlantic craton (M)). There are only single poles, with ages that are not well established, from M and W (Fig. 1), and clearly many more data are required. The Slave–Superior comparison is better documented, and the excellent agreement between poles for the Big

Spruce Complex (BS) and the Otto Stock (OS) which have similar ages, is notable.

The general coherence of the poles implies that these blocks did not move by very large amounts relative to one another during the Hudsonian orogeny. This does not mean that relative movement definitely did not occur, because there are many uncertainties in the palaeomagnetic data. Because of the errors in pole determinations track 5 is not a single line but a broad zone, so that opening and closing of small oceans with dimensions of the order of 1,000 km would go undetected. Moreover, relative motion in a purely longitudinal sense cannot be detected palaeomagnetically; that is, if the Eulerian pole defining the relative motion of two land masses coincides with the palaeomagnetic pole then the relative motions will have no palaeomagnetic expression. If, for example, relative motion of the Slave and Superior Archaean blocks had occurred it would not be seen palaeomagnetically if the motion had been in a longitudinal sense relative to the palaeomagnetic pole. The presence of a single polar track does not therefore necessarily imply that there has been no relative motions among the Archaean cratons; it simply means that the hypothesis of no motions is the simplest but not the only explanation of the results.

The evidence of Fig. 1 suggests that from about 2,200 to 1,800 Myr BP the Laurentian Shield moved at rates comparable with present-day plate motions. The approximate coherence of the polar array indicates that the relative movements among the Archaean cratons were at least several times less than the movement of the whole relative to the pole (100°). It is therefore to be expected that marginal deformation akin to Phanerozoic plate tectonics occurred, and the Coronation geosyncline and the associated Great Slave aulacogen is probably of this nature[25]. During this time linear basins were formed within the Laurentian Shield, and they became filled with deposits. These deposits were deformed in the Hudsonian orogeny, which is related in time to the orogeny that affected the Coronation geosyncline. Thus the Laurentian Shield seems to have undergone intense internal deformation but without loss of general coherence; it does not seem to have been as rigid as the continental parts of modern plates.

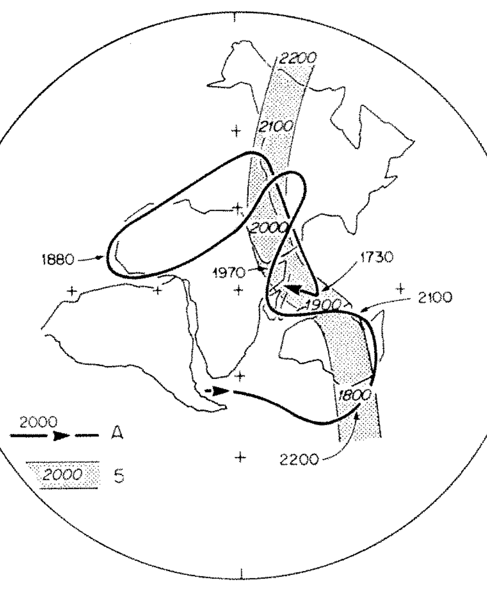

Fig. 2 Polar tracks for interval 2,200 Myr BP to 1,800 Myr BP from West Gondwanaland (A) and Laurentia (5) compared. The continental reconstruction and polar path (A) is from refs 27 and 28. Track 5 is from Fig. 1.

This discussion regarding the nature of Hudsonian orogenesis is consonant with that of Sutton and Watson[26] and the evidence of Fig. 1 seems to confirm partly, if only in tentative fashion, their hypothesis.

It has been suggested[27,28] that a common Proterozoic polar path could be reconstructed for West Gondwanaland (Africa and South America) and Laurentia, so that these cratons formed a single continent from about 2,200 to 1,000 Myr BP. This path and continental reconstruction is shown in Fig. 2 (polar path A). Track 5 is superimposed and there is marked disagreement. The poles from the Otto Stock and Big Spruce Complex (poles OS and BS of Fig. 1 at the 2,200 Myr calibration point of track 5 in Fig. 2) are about 90° away from poles of similar age (Tarkwaian intrusions and Obuasi greenstone[29] near to the 2,200 Myr calibration point of polar path A of Fig. 1) from Africa. We conclude that the palaeomagnetic evidence, as it is known at present, does not support the idea of a Proterozoic supercontinent comprising Laurentia and West Gondwanaland in the interval 2,200 to 1,800 Myr BP. Regarding the palaeomagnetic evidence for the existence of a supercontinent during the later Proterozoic, especially in the interval 1,400 to 1,000 Myr BP for which the evidence has been deemed the strongest[27,28], it is noteworthy that other reviewers[30,31] have argued that the polar curves for this interval from North America and Africa cannot be satisfactorily superimposed, and the critical evidence cited in favour of such a reconstruction[27,28] has not yet been published.

We thank Jean Roy and the referee for comments.

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Received February 25; accepted April 16, 1975.

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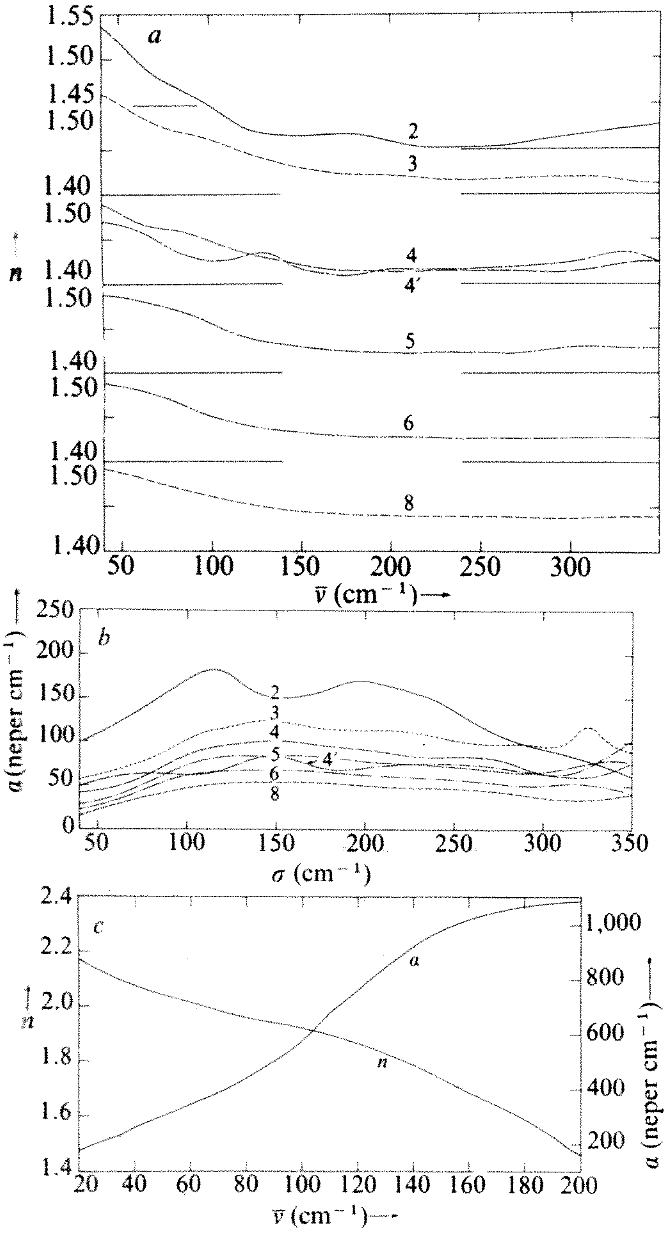
Dispersion of alcohols and water in the submillimetre waveband

FOURIER spectroscopy using polarisation[1] and cube[2,3] Michelson interferometers has made possible the study of the dispersion of aliphatic alcohols[4,5] and water[6] in the submillimetre waveband of the electromagnetic spectrum. Table 1 enumerates measurements we are reporting of refractive index n and absorption coefficient α; the most significant new alcohol and water data are displayed graphically in Fig. 1. The alcohol absorption bands seem to shift in frequency and broaden somewhat as the chain length increases. Alcohols show a monotonic fall of refractive index between 20 and 150 cm^{-1}, but at higher frequencies it remains substantially frequency invariant. The individual dispersions arising from each absorption band cannot easily be discerned.

The absorption function for *tert*-butyl alcohol is different, presumably because the degree of chain polymerisation is uniquely small; the additional band at 70 cm^{-1} is probably caused by Poley absorption in the liquid which consists principally of monomers and dimers.

In the case of water, it has been proposed[6] that the monotonic fall of refractive index can be understood in terms of dielectric relaxation and that the absorption bands are superposed on a Debye relaxation. It is useful to carry out the same exercise on aliphatic alcohols as was carried out for water: calculation from the present data of the complex permittivity function $\hat{\varepsilon}(\omega)$, and subtraction of the contributions to $\hat{\varepsilon}$ from the principal relaxation, calculated using radiofrequency data from Garg and Smyth[7] and others. Corrected $\hat{\varepsilon}(\omega)$ for water, ethyl alcohol and 1-butanol are presented graphically in the form $\varepsilon''(\varepsilon')$ in Fig. 2. In this representation a Debye relaxation appears as a semicircle on the ε' axis, ranging from the principal relaxation value ε_∞, 0 to the optical value n_D^2, 0. Absorption bands appear as arcs of smaller circles ("curlecues") superposed at the high frequency end of the semicircle. The errors in ε'' arising from inaccuracies in the calculation of the contribution from the principal relaxation time are difficult to estimate adequately; but they may be as large as ±30% when ε' is close to $\varepsilon_{\infty 1}$, falling to less than 1% when the maximum value of ε'' is reached.

Fig. 1 Measured optical constants at 20 °C of water and primary aliphatic alcohols as functions of wavenumber $\tilde{\nu}$. Numbers indicate numbers of carbon atoms in molecule; for example curves 4 are for 1-butanol; curve 4′ is for *tert*-butyl alcohol. *a*, Alcohol refractive indices *n*; *b*, alcohol absorption coefficients α; *c*, absorption coefficient and refractive index of water.



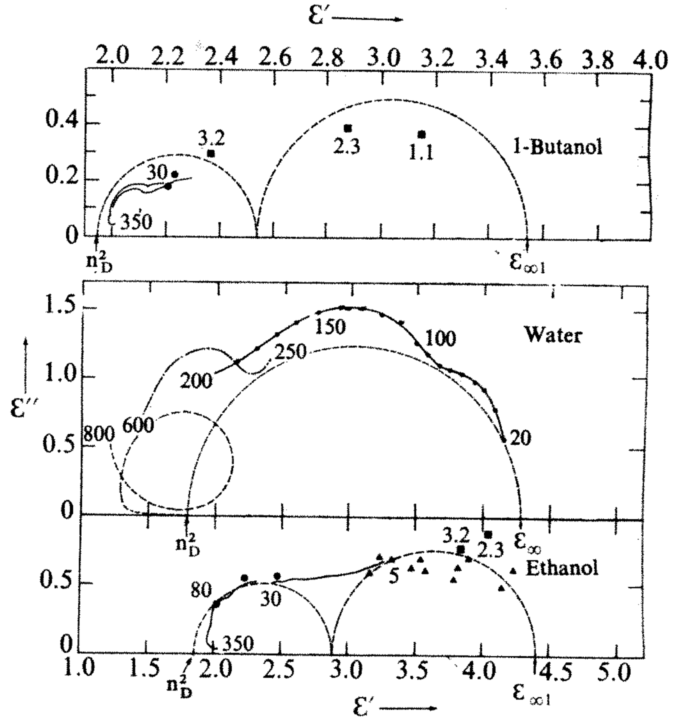


Fig. 2 ε″ (ε′) representations of permittivities of 1-butanol, water and ethanol with contributions from the principal relaxation processes subtracted. Numbers represent wavenumbers in cm^{-1}. ———, ●, Our data; –·–·–, data of ref. 7; ■, data of ref. 8; ▲, other unpublished data.

The new data for water cover a wider frequency range than earlier[6] data, and are of improved accuracy: the discrepancy[6] between ε_∞ calculated from the principal relaxation and that calculated from the proposed high frequency relaxation disappears in the present data. The recent data of Curnutte and Williams[8] from 250 to 800 cm^{-1} are included in Fig. 2, but their absorption coefficients are not shown in the region below 250 cm^{-1}, where they are higher than the present data; the interpretation as a sum of relaxation and resonance processes is not invalidated by this discrepancy.

Alcohols have been supposed[7] to exhibit two separate relaxation processes in the millimetre wave region; to test the validity of this proposal trial semicircles have been drawn, from ($\varepsilon_{\infty 1}$, 0) to ($\varepsilon_{\infty 2}$, 0) and from ($\varepsilon_{\infty 2}$, 0) to (n_D^2, 0). For ethanol there is sufficient microwave data for this interpretation to appear possible, but the situation in the other cases, of which 1-butanol is a typical example, is far from clear, and there is a need for further millimetre wave data before such a proposal can be evaluated. It would be quite possible to represent the 1-butanol data and even those for ethanol by a spread of relaxation times.

Alcohol molecules have been considered[9] to be chain-linked by hydrogen bonds, with principal relaxation activation enthalpy sufficiently large for hydrogen bond breakage. A comparison of the present ethanol data with the low temperature data of Hassion and Cole[9] shows that the activation

Table 1 Refractive index and absorption coefficient data

Wavenumber range (cm^{-1})	Measured quantity	Technique	Author	Water	Methanol	Ethanol	Propanol	*n* Butanol	*tert*-butyl alcohol	Pentanol	Hexanol	Octonal
20–100	α	Melinex beam divider Transmission technique Variable thickness cell Quartz window	GJD			×	×	×	×	×	×	×
20–100	α	Melinex beam divider Transmission technique Fixed thickness cell Quartz window Reflection losses not computed, α expected to be low	GJD	×	×							
20–120	*n*	Melinex beam divider Subtraction technique[4,12]	MNA			×	×	×		×	×	×
20–220	*n*	Polarisation interferometer	MNA					×	×			
5– 30	α, *n*	Polarisation interferometer	MNA		×	×						
20–350	α	Melinex beam divider, quartz and Rigidex window Transmission technique, Variable thickness cell	MSZ			×	×	×	×	×	×	×
29.7	α, *n*	HCN laser, transmission technique	MSZ	×	×	×	×	×		×	×	×
40–350	α, *n*	Melinex beam divider Subtraction technique Improved computation from dispersive interferogram	MNA			×	×	×	×	×	×	×
20–200	α, *n*	Reflection technique Melinex beam divider TPX window	MNA	×								
85	*n*	H_2O laser, reflection technique	MSZ	×	×	×	×	×		×	×	×
85	*n*	H_2O laser, transmission technique	MNA, MSZ				×	×		×	×	×

enthalpy of the first high frequency relaxation process is similar to that for the principal relaxation; this agrees with their proposal that these two processes correspond to the "symmetrical" and "unsymmetrical" hydrogen bond mechanisms also proposed for water[10, 11].

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^{210}Po and ^{239}Pu, ^{240}Pu in biological and water samples from the Bikini and Eniwetok atolls

MEASUREMENTS of the radioactivity in water and biological samples from Bikini and Eniwetok lagoons indicate that although the samples were collected from the most plutonium-polluted waters of the world, the values of the naturally produced radionuclide, polonium-210 were usually greater than the values of plutonium-239 and 240, which are produced by nuclear detonations by factors as great as 100.

With the advent of nuclear testing at the Bikini and Eniwetok atolls in the central Pacific, an environmental laboratory became available for investigating the concentrations of many radionuclides in the marine environment and their uptake by biota. Over the 16 years since the conclusion of nuclear testing, the long lived radionuclides created during the tests and deposited in sediments have had time to approach equilibrium with biota and with natural radionuclides present.

It is of particular interest to compare the concentrations of natural radionuclides, such as ^{210}Po, with concentration of ^{239}Pu and ^{240}Pu produced by nuclear bombs, in that part of the world which has been subject to most radioactive fallout. Both radionuclides decay by α-particle emission of approximately equal energy and both become concentrated in biota. The amount of plutonium to be used in nuclear power reactors is expected to increase by a factor of 1,000 in the next 30 years[1] and with this increased use comes the possibility of accidental or planned releases of plutonium

Table 1 ^{210}Po, ^{239}Pu and ^{240}Pu values in biological and water samples, and the corresponding concentration factors for samples collected in October, 1972 at Bikini and Eniwetok atolls

	Biological samples			Water samples			Concentration factor	
Species and tissue	Location	^{210}Po range* (d.p.m. g^{-1}, wet)	^{239,240}Pu* (d.p.m. g^{-1}, wet)	Location	^{210}Po (d.p.m. m^{-3})	^{239,240}Pu (d.p.m. m^{-3})	^{210}Po range	^{239,240}Pu
Turtle liver (1)†	Eniwetok rubbish dump dock	0.20– 0.69	0.200	Runit dock	188 ±40	190 ±11	$(1.1–3.7) \times 10^3$	1.1×10^3
Goatfish viscera (6)	Eniwetok, Runit seaward reef	2.60– 9.27	0.650	200 yards off Runit	146 ±20	190 ±11	$(1.8–6.3) \times 10^4$	3.4×10^3
Wavyback skipjack (1), dark muscle	Eniwetok Lagoon off Runit	2.50– 8.94	0.280	,, ,,	146 ±20	190 ±11	$(1.7–6.1) \times 10^4$	1.5×10^3
Mullet (2) eviscerated whole	Eniwetok, Runit seaward reef	0.50– 1.90	0.003	,, ,,	146 ±20	190 ±11	$(3.4–13) \times 10^3$	16
Surgeon fish (3), bone	Bikini *a*–6	8.15–49.70	<0.001	*b*–15	107 ±10	73 ±9	$(7.6–46.0) \times 10^4$	<20
Surgeon fish (3), muscle	Bikini *a*–6	0.02– 0.15	<0.001	*b*–15	107 ±10	73 ±9	$(1.9–14.0) \times 10^2$	<20
Surgeon fish (1), eviscerated whole	Bikini *a*–2	0.40– 2.50	0.018	*b*–2	124 ±13	138 ±9	$(3.2–20.0) \times 10^3$	130
Surgeon fish (3), viscera	Bikini *a*–6	0.51– 3.09	1.330	*b*–15	107 ±10	73 ±9	$(4.7–29.0) \times 10^3$	1.8×10^4
Convict surgeon (3), muscle	Bikini *a*–3	0.10– 0.59	<0.001	*b*–6	125 ±13	51 ±7	$(8.0–47.0) \times 10^2$	<20
Convict surgeon (17), muscle	Bikini *a*–7	0.06– 0.34	<0.001	*b*–18	145 ±14	102 ±9	$(4.1–23.5) \times 10^2$	<20
Convict surgeon (13), muscle	Bikini *a*–4	0.13– 0.77	<0.001	*c*–12	135 ±14	45 ±5	$(9.6–57.0) \times 10^2$	<20
Convict surgeon (6), muscle	Bikini *a*–5	0.09– 0.56	<0.001	*b*–10	153 ±15	7 ±2	$(5.9–36.5) \times 10^2$	<20
Convict surgeon (13), eviscerated whole	Eniwetok, Runit seaward reef	0.80– 2.75	0.005	200 yards off Runit	146 ±20	190 ±11	$(5.5–19) \times 10^3$	26
Convict surgeon (21), eviscerated whole	Eniwetok, Runit	0.20– 3.31	0.025	,, ,,	146 ±20	190 ±11	$(1.4–22.6) \times 10^3$	130
Convict surgeon (4), eviscerated whole	Bikini *a*–2	1.40– 8.28	0.028	*b*–2	124 ±13	138 ±9	$(1.1–6.7) \times 10^4$	200
Convict surgeon (1), eviscerated whole	Bikini *a*–1	0.46– 2.82	0.010	*c*–3	141 ±15	138 ±9	$(3.3–21.7) \times 10^3$	72
Convict surgeon (4), eviscerated whole	Bikini *a*–1	0.34– 2.06	0.017	*c*–3	141 ±15	138 ±9	$(2.4–14.6) \times 10^3$	120
Convict surgeon (17), vliver	Bikini *a*–7	2.04–12.50	0.913	*b*–18	145 ±14	102 ±9	$(1.4–8.6) \times 10^4$	9×10^3
Convict surgeon (21), iscera	Eniwetok, Runit seaward reef	1.20– 4.20	0.140	200 yards off Runit	146 ±20	190 ±11	$(8.2–28.7) \times 10^3$	7.4×10^2
Convict surgeon (13), viscera minus liver	Bikini *a*–4	1.67–10.21	0.350	*c*–12	135 ±14	45 ±5	$(1.2–7.6) \times 10^4$	7.8×10^2

*Analytical error is ±10% for polonium and ±20% for plutonium.

†Numbers in parentheses indicate the number of fish or organs combined in a given sample. Concentration factors are calculated as a ratio of activity per gram of wet tissue to activity per gram of water.

into the food chain of man. The uptake of plutonium by biota in the marine environment is not well understood and a full evaluation of their potential hazard cannot yet be made.

Natural waters contain ^{210}Po as a member of the ^{238}U decay chain. Atmospheric aerosols also contain ^{210}Po which are scavenged[2] by precipitation and enter the hydrosphere. Once in the aquatic environment, polonium and plutonium undergo processes such as precipitation, redistribution, and changes in physical–chemical and oxidation states which may not be similar for the two elements. Both, however, are apparently available biologically as they are accumulated by algae, phytoplankton, zooplankton, vertebrates and invertebrates[3–8].

The samples discussed here were collected by the Laboratory of Radiation Ecology (LRE) during the 1972 joint expedition with the Puerto Rico Nuclear Center and the Lawrence Livermore Laboratory to Bikini and Eniwetok atolls. The locations of the collecting stations are shown in Fig. 1. The analytical results and collection locations of both biological and water samples, and the number of individual species of biological samples measured, are given in Table 1. Samples labelled as 'eviscerated whole' are whole fish which have had the viscera (liver, kidney, gonads, gut contents, and gastrointestinal tract) removed.

As several months elapsed between the collection and analysis of samples, the disintegration rate of ^{210}Po was corrected to allow for decay between the date of counting and the date of collection. The lower and upper limits given in Table 1 indicate the possible range of values if, first, ^{210}Po was derived entirely from the decay of ^{210}Pb in the sample (the lower limit) or, second, no ^{210}Pb was present and the ^{210}Po measured was the true concentration present at the sampling time (the upper limit). The real value is expected to be near the upper limit for most samples since, in pelagic fish, the ^{210}Po is obtained by direct uptake and not as much by ingrowth from ^{210}Pb; measurements showed 1–5 times more ^{210}Po than ^{210}Pb (refs 8–10).

Table 1 shows that ^{210}Po activity is highest in bone, decreasing in order in liver, viscera and muscle. Although bone and liver have been reported as the major repositories for plutonium[10], the plutonium content of the single bone samples reported was at the detection limit of 0.001 d.p.m. per g, wet weight. The high concentrations of both ^{210}Po and ^{239}Pu and ^{240}Pu in viscera and viscera minus liver (convict surgeon), together with the fact that algae in the diet of these species concentrates those radionuclides from seawater, seems to support the idea that both radionuclides enter the fish during the ingestion of food.

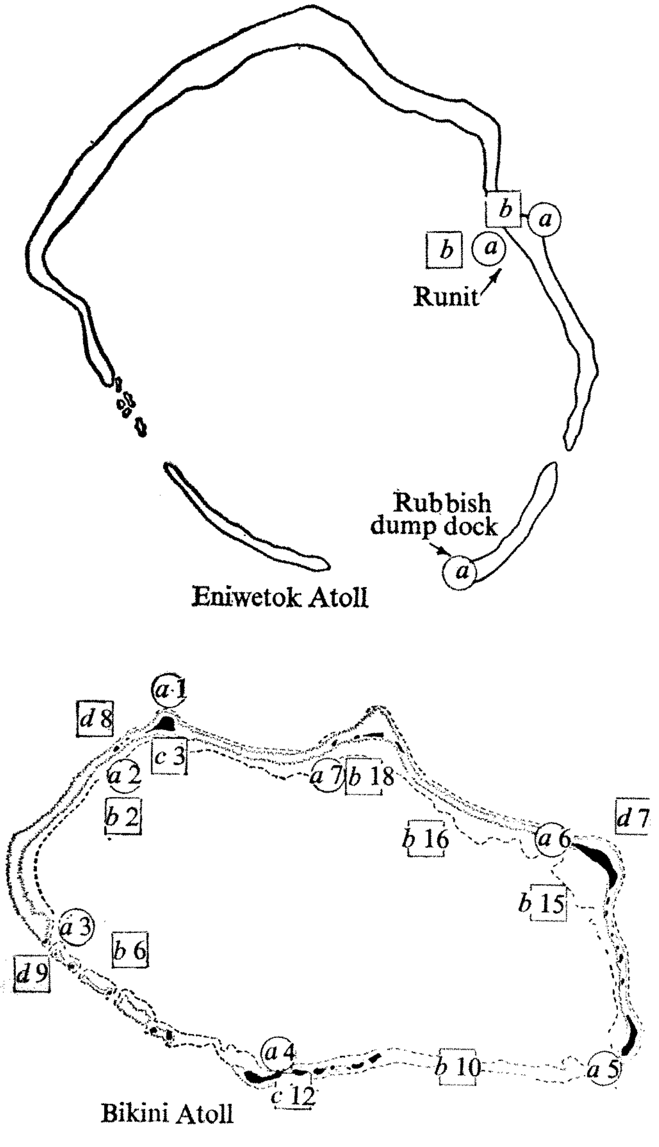
Values of plutonium radioactivity have been reported in a large number of biological samples from Eniwetok Atoll[11]. These data also show that the highest plutonium concentrations occur in the viscera of fish. The food contents of the digestive organs were not separated, however, so it is not possible to estimate what fraction of the activity is associated with food and how much occurs in the tissues or organs of the fish.

The convict surgeon and surgeon fish were selected for ^{239}Pu, ^{240}Pu and ^{210}Po analysis because they are local feeders and would concentrate radionuclides from nearby waters.

The concentrations of ^{239}Pu, ^{240}Pu and ^{210}Po measured in water from near the sites of the fish collection were used to determine the concentration factors shown in Table 1. The ^{210}Po concentration in the water was calculated from the ^{210}Pb concentration measured in the samples after most of the ^{210}Po had decayed. The error on the ^{210}Po calculation is expected to be small, as the ratio of ^{210}Po/^{210}Pb is approximately 1 : 2 in seawater[8,9]; thus, the ^{210}Pb and ^{210}Po would be near secular equilibrium in the marine environment at Bikini and Eniwetok atolls. The highest ^{239}Pu and ^{240}Pu contents in the water column were found in areas where the highest activity of those radionuclides occurred in sediments, as reported already for Bikini[12]. The highest ^{210}Po concentration was found outside the lagoon in the deep ocean waters, and one possible explanation could be that biological uptake results in a shorter resident time of ^{210}Po–^{210}Pb in the lagoon. The concentration of ^{210}Pb in the particulate phase, (>0.3 μm in size) was less than 10% of the total; the concentrations of ^{239}Pu and ^{240}Pu in the particulate phase varied from 2 to 60% of the total depending on where it was collected from[12]. The overall result indicates that inside the lagoon the radioactivity values of plutonium were more variable than those of ^{210}Po.

Concentration factors of ^{210}Po, ^{239}Pu and ^{240}Pu in organisms (Table 1) are calculated using the total concentrations (particulate plus soluble) measured in the water. All specimens measured have concentration factors for ^{210}Po that are greater (as much as 100 times greater) than those for ^{239}Pu and ^{240}Pu with the exception of samples of turtle liver and surgeon fish viscera. Although these samples were collected in the most plutonium-polluted waters of the world we conclude that the plutonium radioactivity is only a fraction of the natural polonium radioactivity, and that a greater radiation dose to the marine organisms of Bikini and Eniwetok lagoons would be received from ^{210}Po than from ^{239}Pu and ^{240}Pu. This conclusion should not, however, lead to neglect in studies of plutonium concentrations in the higher trophic levels and of its transport through the food web to man.

Fig. 1 Sampling stations at Bikini and Eniwetok atolls. *a*, Intertidal zone, biological stations; *b*, lagoon stations; *c*, crater stations; *d*, ocean stations.



This work was carried out under joint contract from the US Atomic Energy Commission and the University of Washington.

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Received March 3; accepted April 4, 1975.

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r and K selection in kangaroos

MacArthur and Wilson[1] used the terms 'K selection' and 'r selection' to describe two general kinds of selection they believed could be functioning in nature. Where there is no crowding, evolution favours productivity (r selection). Alternatively in a crowded situation where some resource is scarce, evolution favours the efficient use of this resource in the production of offspring (K selection). Thus r selection occurs when it is more important to increase numbers and K selection when population size is near the maximum that can be carried in the prevailing environmental conditions. Pianka[2] summarised a number of theoretical correlates of r and K selection, and some of these are given in Table 1.

Reports on natural situations in which r or K selection can be expected to have occurred in closely related organisms are quite rare in the literature and here I describe one such example. The kangaroos and wallabies of Australia form a cohesive group with a relatively recent origin[3]. The amount of genetic divergence between species is fairly limited since viable, though infertile, hybrids are found between many members of the group[4]. The animals occur in a wide range of habitats, however, including the extremely unpredictable low rainfall environment of central Australia and the more stable environment of the eastern forests. The red kangaroo (*Macropus* (*Megaleia*) *rufa*) is found in the former and the eastern grey kangaroo (*Macropus* (*Macropus*) *giganteus*) in the latter[3]. These two similarly sized species provide an interesting comparison because the red kangaroo should be subject to predominantly r selection and the eastern grey kangaroo to predominantly K selection. The revelant ecological data for these two species are summarised in Table 1.

The fit of the data to those suggested on theoretical grounds is quite good. The red kangaroo is clearly adapted

Table 1 Summary of some theoretical correlates of r and K selection and relevant data on the red and eastern grey kangaroos

Item	r selection		K selection	
	Theoretical[2]	Red kangaroo	Eastern grey kangaroo	Theoretical[2]
Climate	Variable or unpredictable	Arid zone (unpredictable)	Forest (more constant than for red kangaroo)	Fairly constant or predictable
Mortality	Catastrophic, non-directed, density independent	Catastrophic (drought) Depending on conditions young leaving pouch either all die or almost all survive	Rarely catastrophic. Normally 50% of young leaving pouch die by 15 months*	Fairly constant, at or near carrying capacity
Intraspecific competition	Lax	Population usually well below carrying capacity	Populations near carrying capacity*	Keen
Interspecific competition	Lax	Sympatric with three other large macropodids	Sympatric with eight other large macropodids	Keen
Selection favours:	Rapid development	Gestation[3] 33 d, pouch life[3] 235 d, suckle until 14 months of age†	Gestation[4] 36 d, pouch life[4] 319 d, suckle until 18 months of age†	Slower development
	High r^{max}	Continuous opportunist breeder[3], delayed blastocyst[3] (In drought, discard pouch young while small and replace, maintaining mother's condition[5])	Usually seasonal breeder[3], partially delayed blastocyst[3] (In drought carry pouch young until mother almost *in extremis**)	Lower resource threshold
	Early reproduction	As soon as conditions permit after reaching 18 months of age†	When good conditions occur at the beginning of the first breeding season after reaching 18 months of age (often wait, 1, 2 or more years)†	Late reproduction
	Mobile‡	Mobile in bad seasons[3]	Sedentary[3]	Sedentary‡

*T. Kirkpatrick (personal communication).
†W. E. Poole (personal communication).
‡B. S. Richardson (personal communication).

for a rapid population recovery following a bad drought, whereas the grey kangaroo is adapted to producing young best able to enter the adult population in the face of competition for limited resources. In this sense the pattern seen in the grey kangaroo does not fit that originally described for K selection. Here the pressure is not on the mother to find sufficient of the limiting resource to produce a youngster ready to enter the population but on the youngster, on permanently leaving the pouch to make a place for himself in the adult population. Thus in the original forest habitat it seems likely that less than 10% of eastern grey kangaroos leaving the pouch survive the next 6 months (T. Kirkpatrick, personal communication). In this case K selection involves production of a young animal best able to fight for its place, rather than efficiency of the mother to convert the resources into young (that is, economy). It seems likely that the patterns suggested by Pianka[2] for K selection when energy is the limiting factor will not necessarily be satisfactory for other resources, for example, when space is the limiting factor. In the grey kangaroo selection may favour the doe who is quite profligate with her time and energy (not limiting) if this increases her offspring's chance of survival over a doe who produces more young. It would depend on the role chance plays in the choice of the survivor.

Note that since the European settlement of Australia the eastern grey kangaroo has extended its range many hundreds of miles westward into the dry inland zone occupied by the red kangaroo. This seems to be at least partly caused by the supply of stock watering points and the more predictable nature of this environment which results. The red kangaroo has an advantage over the eastern grey kangaroo in this area only during extended severe droughts (ref. 3 and T. Kirkpatrick, personal communication). Also the numbers of both species of kangaroo in this area have increased greatly this century[3] and, if the species is in direct competition for a limited resource, the eastern grey kangaroo should be at an advantage. Culling by professional hunters may, however, stop the populations reaching any resource limit in these areas.

I thank Dr T. Kirkpatrick and Mr W. Poole for permission to quote unpublished data, and Dr J. Dearn, Dr D. Dykhuizen and Professor B. John for comments on the manuscript.

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Received February 24; accepted April 7, 1975.

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Effect of simulated 'acid rain' on cation loss from leaves

INCREASING consumption of fossil fuels over the past two decades has given rise to elevated levels of nitrogen and sulphur oxides in the atmosphere. These compounds undergo hydrolysis and oxidation giving rise to nitric and sulphuric acids. The presence of these compounds in precipitation may enhance its hydrogen ion concentration[1]. Normally, precipitation has a *p*H range of 6.5–7.0 but the trend towards increasing acidity is increasingly clear. *p*H values of 4.0 have been reported for Cumbria, UK[2], 3.8–4.2 for Liverpool, UK (M. H. Eastwood, personal communication), approaching 3 for Hubbard Brook, New Hampshire[1], and a value of 2.8 has been recorded in Scandinavia[1]. One of the main effects of rainfall on plants is the leaching of nutrients from leaves. What effect rainfall of low *p*H may have on this process is at present unknown, as previous leaching studies[3] have used distilled water at *p*H 6–7.

Little attention has been paid to the effects of 'acid rain' on vegetation, possibly as a result of the absence of visible injury at *p*H values similar to those recorded in rainfall. Experiments reported by Wood and Borman[4] on growth of yellow birch seedlings in relation to *p*H of artificial mist, revealed foliar spot and marginal necrosis of leaves at *p*H 2.3. At *p*H levels above this, no foliar injury was reported. Sheridan and Rosenstreter[5] demonstrated a decrease in photosynthetic rate in the moss *Tortula ruraliformis* in response to increasing acidity of rainfall. Concomitant with this was a reduction in the ratio of chlorophyll A/chlorophyll B, and a sharp decline in respiration rate below *p*H 3. Gordon (cited in ref. 5) demonstrated that the developmental malformation of pine termel 'short-long needle disease' occurred in response to a range of rainfall of *p*H between 1.5 and 4.5.

We investigated nutrient losses from leaves in response to *p*H of rainfall. Tobacco plants (*Nicotiana tabacum* L. var virginiana) of the same age were selected, and leaves at the same state of maturity were used for leaching. One batch was leached with deionised water at *p*H 6.7, whereas the second batch of plants was leached with H_2SO_4 adjusted to *p*H 3.0. Leaching techniques were similar to those used by Tukey *et al.*[3], except that our leaching rates were slightly lower, being maintained at 400 ml h^{-1} over a 6-h experimental period. The relationship of these leaching rates to natural precipitation rates will be dependent on local precipitation data, and as these may vary considerably from area to area, no exact relationship between the laboratory experiment and field precipitation levels can be given. Leached cations were collected on columns of Zerolit 325 cation-exchange resin. Samples of the leaching solution were passed through similar columns with the same rate and time period to measure background cationic levels in the leaching solutions. Cations were eluted from the resin columns using 10% HCl, and eluate cation concentrations assayed for K^+, Ca^{2+} (by flame photometry) and Mg^{2+} (by atomic absorption spectrophotometry). In experiments with 'acid rain' at *p*H 3, a correction factor was calculated for each ion to allow for loss of efficiency of the ion-exchange resin in retention of cations at an elevated H^+ concentration.

Table 1 Cation loss from *Nicotiana* leaves in relation to *p*H of leaching solution

*p*H of leaching solution	K^+	Leached cation Ca^{2+}	Mg^{2+}
6.7	58±16	73±18	3.4±1.4
3.0	13±4.8	273±66	4.6±2.0
Significance	*	*	—

Cation concentrations are given in µg cation leached per leaf per 6-h wash. All experimental leaves were fully expanded and 8 weeks old. Values represent the means of 21 replicated ± s.e.

*Probability of the two means being equal is less than 0.01. Significance was calculated using a *t* test for groups of equal sizes[6].

Table 1 shows that increases in foliar Ca^{2+} loss occur in response to a decrease in rainfall *p*H, whereas losses in K^+ decrease. Little change occurs in levels of Mg^{2+} lost. Mecklenburg *et al.*[7] demonstrated that Ca^{2+} loss from leaves occurs by a passive process of ion exchange, in which H^+ in rainfall exchange with foliar Ca^{2+}, resulting in the loss of the latter. Consequently, Ca^{2+} losses from leaves will be solely dependent on H^+ concentrations in rainfall. This agrees well with the data in Table 1, in which increased Ca^{2+} loss occurs with increasing H^+ concentration. The decrease in K^+ loss with increasing H^+ concentration is less readily explained. Tukey *et al.*[3] have demonstrated that Ca^{2+} is more readily leached than K^+, using distilled water; as most H^+ may be used to release Ca^{2+}, this could leave fewer to replace K^+.

An alternative explanation is that of physiological damage caused by 'acid rain'. Rains *et al.*[8] have suggested that hydrogen ions may damage the selective uptake mechanism for K^+ in root cells, the integrity of this mechanism being protected by Ca^{2+}. If Ca^{2+} levels are depleted and replaced by H^+, then a subsequent reduction in K^+ uptake could lead to a decrease in K^+ available for leaching. Mecklenberg and Tukey[9] have also demonstrated that the most recently absorbed Ca^{2+} are most readily leached; so in view of the slow upward movement of Ca^{2+} (10 cm d^{-1})[10], the possibility of rapid depletion of freely exchangeable Ca^{2+} is likely. The reason for the increased retention of K^+ in the 'acid rain' regime is certainly elusive, and any explanation is only tentative. Other factors, at present unknown, may be operating to regulate these levels.

These results imply that increasing Ca^{2+} loss may occur from leaves with increase in H^+ content of precipitation. Several questions must be answered before the importance of these observations can be fully evaluated. These include measurements of cation loss from different species in different *p*H regimes, and a characterisation of the form in which Ca^{2+} is lost from leaves. 'Acid rain' contains free SO_4^-, and Ca^{2+} lost from foliage may react with this to form insoluble $CaSO_4$. This could have considerable implications in the natural cycling of this element in polluted ecosystems. Further experiments are in process to clarify these points.

The provision of an SRC research studentship for one of the authors (J.A.W.F.) is gratefully acknowledged.

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Received January 31; accepted April 17, 1975.

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Mate selection in Pacific tree frogs

DARWIN[1] suggested that, in many animal species, females select mates and that some male courtship displays and secondary sex characters are a product of this sexual selection. Although the importance of sexual selection is generally acknowledged[2], only a few studies have shown both that females prefer certain males and also that this preference is a function of male display behaviour, for example, studies of polygamous birds[3] and fruitflies[4]. We have observed that in the Pacific tree frog (*Hyla regilla*), females mate preferentially with certain males, and that this preference is related to differences between males in calling behaviour.

The males congregate during the spring in ponds and lakes where they call, mostly at night, for several months. Within a local area, such as a small pond, males space themselves at least 50 cm apart (C.L.W. and J.R.K., unpublished) and call in bouts. Typically, all males call for a few minutes, then all are silent for a few minutes before calling again. Attracted by the calls[5], females approach individual males, and only when a female nearly touches a male does he cease calling and attempt amplexus. We set out to test the hypothesis, suggested by previous observations of other frogs[6], that females preferentially approach males that initiate bouts of calling.

Foster[7] reported that certain males initiate more bouts than would be expected by chance, and numerous observations of our own verify this. For example, in a 1.5 h period, a group of approximately 20 frogs produced 10 bouts; one individual initiated seven of these, was second to begin calling in two, and third in one. It was not unusual for an individual to initiate bouts for several hours, and occasionally a frog would be a 'bout leader' for several consecutive nights.

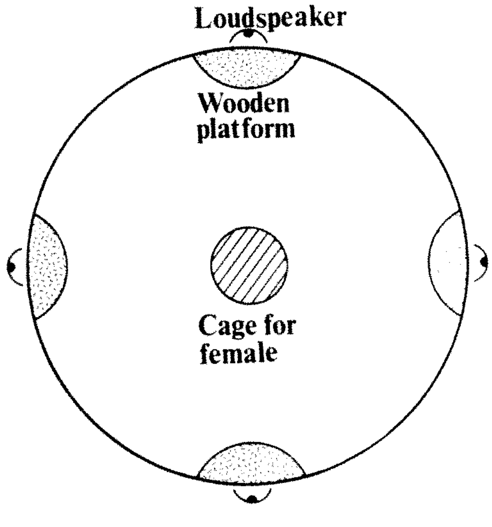
We also discovered, by systematic field observations, that the calling of bout leaders differs in several other ways from that of other frogs[8]. We observed pairs of calling frogs, defining one frog of a pair as a bout leader if it initiated three consecutive bouts. Not only did the bout leaders initiate bouts of calling, but they were the last to stop calling when bouts ended ($P<0.01$); they called at a faster rate during bouts ($P<0.01$); they called louder ($P<0.025$); and they were more likely to call during periods not defined as bouts ($P<0.01$). Thus bout leaders call more actively than other frogs: they expend more energy on calling.

It is rare to see females selecting males in the field, but we observed three instances of this, and in each case the favoured frog was a bout leader of a group of at least five frogs. The probability of this occurring by chance is less than $(1/5)^3=0.008$.

We tested more rigorously the relative success of bout leaders and other males in a laboratory experiment. We did the experiment in a circular arena having four loudspeakers on the circumference (Fig. 1). A Uher 4000 Report-L tape recorder, wired to a stepping relay, played identical calls sequentially (corresponding to the antiphonal calling of real frogs) through the speakers. Each speaker called 30 times per min (approximately the same rate as the frog from which the calls were recorded). Bouts of calling were 2 min long, with 1 min lulls between. A randomly chosen speaker was the bout leader for each trial; for each bout it called three times before the other speakers were turned on (not an unusual number of times for a bout leader to call before other frogs join). We released the female from the cage in the centre of the arena about 15 s after the start of the fourth bout. If she moved, within 60 min, to within 2 cm horizontal distance of a speaker, we recorded a 'choice'.

One would expect, by chance alone, that one-quarter of the responding females would choose the bout leader. Of 18 females, however, 14 choose the bout leader ($P<0.001$; binomial test). Our experiment shows therefore that females prefer bout leaders even when they give the same call as other chorus members. We do not know if the other behavioural differences between bout leaders and other frogs enhance this preference.

Fig. 1 Top view of the arena (1 m diameter) used in the female choice experiment. The arena was filled with water (depth 10 cm), and the cage floor and the platforms were slightly submerged (0.5 cm).



Since it is advantageous for a male to initiate bouts, why does the competition to do this not result in all males calling all the time? What limits males from calling more? Since calling involves repeated and, what seem to be very strong contractions of abdominal muscles[9], we hypothesised that the length of a calling bout is limited by fatigue of these muscles. To test this hypothesis, we assayed ATP and lactate in the abdominal muscles, predicting lower ATP and higher lactate levels in frogs that had just finished a bout of calling compared with controls. We froze experimental animals in liquid nitrogen, cut off the abdominal muscles while still frozen, then extracted the metabolites and assayed them enzymatically by established procedures[10-11]. The results (Table 1) show no significant differences between control and calling frogs. Thus they do not support the hypothesis that frogs stop calling because of muscular fatigue, and the question of why frogs do not call continuously remains unanswered.

Table 1 ATP and lactate (μmol per g wet tissue) in frog calling muscles

Replicate	ATP		Lactate	
	Calling frog	Control	Calling frog	Control
1	1.75	3.05	0.83	0.51
2	2.97	2.90	Nil	2.13
3	2.85	2.74	0.63	3.10
4	Nil	Nil	0.91	4.41
5	Nil	4.41	0.42	0.42

Our study also raises several evolutionary questions. Why do females choose males that call most actively? Is it simply that these males are easiest to locate because they call during periods of little acoustical interference; or do they also make better mates? Since a male's sole investment in the offspring is sperm, we must ask if the sperm of bout leaders produces more or better offspring. Finally, accepting that the amount of calling a male can do is limited and that males compete to call during periods when few others are calling, why do they not develop other strategies of calling? Some males, for example, could rest during bouts, and then start calling when the other frogs stopped. The answer may be that it is advantageous to contribute to bouts, not only because it increases the overall intensity of calling and therefore may attract more females to the area, but also because, in doing so, a frog interferes acoustically with the calling of other individuals, reducing their chances of attracting females.

We thank W. Driedzic for assistance with the biochemical assays, and W. Driedzic and D. Fairbairn for helpful comments on the manuscript. This research was supported by a grant from the National Research Council of Canada to J. R. K.

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Vertical clinging and leaping in a neotropical anthropoid

Napier and Walker[1] proposed a new category of primate locomotor behaviour which they called "vertical clinging and leaping". They believed that among living primates this category included only certain prosimians. It has since been recognised that non-prosimian primates (for example, *Saguinus*[2], *Cacajao*[3], and *Callicebus*[4]) may at times also leap to and from postures in which the body is held vertically at rest. In a recent review of vertical clinging and leaping Stern and Oxnard suggest that "future behavioural observations will show that almost all primates may occasionally move in this way"[3]. In view of the acrobatic arboreal abilities of primates this would not seem remarkable, given appropriate environmental conditions and stimuli. So far, however, no one has reported any anthropoid primate engaging habitually in this form of positional[5] behaviour.

From July to September, 1974, we observed a group of nine pygmy marmosets, *Cebuella pygmaea,* for over 200 h in inundatable forest at the edge of the Nanay river, about 30 km south-west of Iquitos, Peru. The group consisted of four adults, three subadults, and two infants carried by an adult male. (A comprehensive study of this group, including a detailed presentation of methods, is being prepared by M. Ramirez.) The vegetation was virgin tropical rain forest with a continuous canopy between roughly 10 and 20 m high, and a few emergent trees up to 35 m. Relatively heavy undergrowth existed below the canopy, including many bushes and lianas of varying size. The marmosets were normally awake for about 11.5 h each day, from 0615 to 1745. By quantifying frequency and duration of activity patterns for 4 d, we found that adult animals spend approximately 48% of their time feeding (including foraging for insects), 12% moving (exclusive of locomotion involved in feeding), and 40% resting (including grooming). We obtained the following data on adult animals while noting whether the substrate was more or less horizontal (less than 45° inclination) or vertical (greater than 45°). During 16 h observation of feeding behaviour, 77% of the animals' time was spent clinging to vertical supports of large diameter while feeding on sap. *Cebuella* used a wide range of locomotor behaviours, including quadrupedal walking and running along supports as well as leaping between supports. We observed leaps of up to 2 m which confirms the observations of Christen on captive *Cebuella*[6]. We observed and counted 288 leaps over several days and of these 38% were from one vertical support to another, 28% from a horizontal to a vertical, 19% from a vertical to a horizontal, and only 15% were from a horizontal to a horizontal. Thus, a total of 85% of all leaps observed were to and/or from a vertical supporting branch. Animals spent 57% of their resting time (48 h observation) clinging to vertical supports. The longest period during which an adult individual clung continuously to a vertical support without moving was 96 min; the longest period that an animal was observed sitting on a horizontal support was 16 min. We believe these data amply justify consideration of *Cebuella pygmaea* as a neotropical vertical clinger and leaper.

Cebuella is a tiny animal, the smallest New World monkey and the second smallest primate; average weight of adults is 145 g (ref. 7). It spends most of its feeding time obtaining and eating sap, a behaviour which has also been noted for *Cebuella* in Columbia by Moynihan[8]. In addition *Cebuella* feeds on insects and berries. Insects and spiders are approached by slow stalking and are frequently captured using a rapid pouncing leap. Most insect foraging occurs in areas of entangled lianas and bushes below the level of the closed canopy (the primary foraging zone). This substrate, in contrast to the canopy, has a relatively high frequency of vertical supports. An individual may spend 85 to 90% of its total waking hours in the primary foraging zone. Berries ('renaco', *Ficus* sp., and 'caymittillo') are an infrequent source of food, and are procured from terminal branches in the canopy (that is, a secondary foraging zone), or from bushes below it.

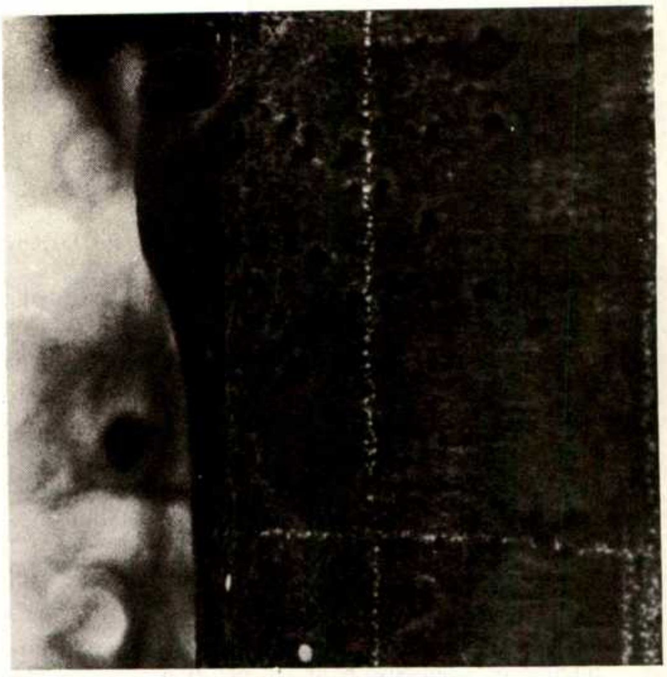

Fig. 1 Posture of adult *Cebuella* while feeding on sap of *Quararibea* sp. (Bombacaceae).

In terms of time spent, sap was the most important food resource for *Cebuella* during our study. Sap was obtained from lianas and from the trunks of trees of both large and small diameter. *Cebuella* obtained this exudate by using its semiprocumbent lower incisor teeth to scrape fissures into the bark, primarily while clinging to large vertical tree trunks. The trunk diameter of the preferred sap-feeding tree (*Quararibea* sp., Bombacaceae) measured 51 cm at the feeding level. Adult individuals spent an average of 97 min each day preparing, and/or feeding at, sap-holes on this large tree alone. The sap of other large diameter trees, including *Palicourea macrobotrys* (Rubiaceae), was also used for feeding. Throughout these feeding bouts on large tree trunks a variety of clinging postures were used, with the body oriented upright, inclined, or head downward. In the latter posture the lower extremity was rotated laterally. In the upright position the base of the tail was often pressed to the tree trunk; the forelimbs were either abducted and fully extended (Fig. 1) or adducted at the shoulder and partially flexed at the elbow; the hindlimbs were widely abducted indicating great hip mobility, or closely adducted and flexed beneath the body. In all these postures the claws of *Cebuella* served a particularly important function specific to feeding. During scraping actions of the teeth against the bark, the manual claws anchored the forelimbs laterally, while the pedal claws anchored the hind limbs below (Fig. 1). The head (and often the shoulders) was fixed on the vertical column. The forelimbs acted as levers, flexing at the elbow and/or wrist, increasing the mechanical advantage at the mandibular incisors. Most important, during all postural behaviours on large substrates the claws seemed to be firmly embedded in the bark.

Napier and Walker[1], in recognising vertical clinging and leaping as a distinct locomotor category, suggested a fairly precise correlation between its behavioural and morphological features. Stern and Oxnard[3] pointed out, however, that multivariate analyses of the various components of the locomotor skeleton "suggest that there is more than one kind of morphology among vertical clingers and leapers and, therefore, that there is likely to be more than one kind of vertical clinging and leaping." They distinguished two such kinds, one for the indriids, and a second for *Microcebus, Galago,* and *Tarsius*; subsequently Oxnard[10] suggested a third mode manifested by *Lepilemur*. Jouffroy and Gasc[11] distinguished the same three locomotor groups based on morphology presumed to be adapted specifically for leaping and not for clinging.

Our data indicate that claws in *Cebuella* are for clinging to large vertical supports, and are not part of a complex related to leaping. We do not yet know what is the adaptive relationship between leaping and vertical clinging; *Cebuella* does both.

In *Cebuella* vertical clinging involves grasping (of slender supports) with the hallux, but also involves clinging to broad supports with the claws of both hands and feet. As Cartmill has pointed out, clawed primates are generally more competent than clawless ones of similar body size in moving on thick vertical supports. Vertical positional behaviour offers the pygmy marmosets their only (or at least their most efficient) access to sap, an apparently essential food resource. Clinging is a postural not a locomotor adaptation. Failure to distinguish between posture and locomotion may overlook the biological importance[12] of clinging which in *Cebuella* is clearly related to feeding. Differing biological functions for vertical clinging have probably evolved in different primate lineages. Clinging and leaping faculties may or may not be part of the same form–function complex[12], the observed differences in morphology arising from this.

We conclude that in *Cebuella,* semiprocumbent mandibular incisors, small body size and clawed digits are part of an adaptive complex especially related to feeding on sap while clinging to large vertical supports. Similarly, *Galago elegantulus*[13], *Phaner furcifer* and *Microcebus coquereli*[14], of medium body size, with very procumbent mandibular incisors, and modified nails which function as claws, also feed on sap while clinging to large tree trunks[15]. Thus, there is an evolutionary convergence in biological importance (not in diagnostic vertical clinging and leaping morphology) of vertical clinging adaptations to exploit sap-feeding niches in certain medium-sized nocturnal galagines and cheirogaleines, and in the small diurnal *Cebuella pygmaea.*

This suggests that vertical clinging behaviours are postural adaptations, related to feeding and dependent on the size of the support and on the spatial location of the food resource. Leaping behaviours, on the other hand, are locomotor adaptations. Thus, they serve different biological functions which may or may not be related to obtaining food.

We thank Curtis Freese, Consultant, Pan-American Health Organization, for locating and arranging for the study site, Juan Revilla for identification of trees, and Suzanne Ripley for comments on the manuscript. This work was supported by grants from the City University of New York and from the Explorers Club to W.K.

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Control of rust diseases by diterpenes from *Nicotiana glutinosa*

AN epimeric mixture of the diterpenes sclareol and 13-epi-sclareol (sclareol) (Fig. 1) has been shown to constitute 10% of the surface exudate on leaves of *Nicotiana glutinosa*[1]. Investigations into its effect on fungal growth showed that it neither inhibits the spore germination of several species of facultative pathogens *in vitro* nor reduces the weight of mycelium produced in liquid medium. The development of hyphae of certain species of fungi, for example *Alternaria brassicicola,* is, however, markedly affected by this compound. In the presence of sclareol, increased branching is induced which leads to a substantial reduction in the diameter of colonies growing on agar[1]. Such morphological effects could be interpreted as the result of alterations in the regulation of natural fungal growth processes. This report describes the effect of sclareol on the germination and growth of rust fungi *in vitro,* and the protection it can provide to plants against diseases caused by these fungi.

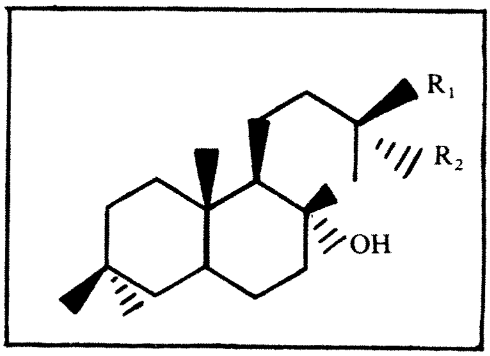

Fig. 1 Sclareol: an epimeric mixture of sclareol ($R_1 = OH{:}R_2 = Me$) and 13-epi-sclareol ($R_1 = Me{:}R_2 = OH$).

The germination of rust uredospores was assessed in the well slide test[2] and on the surface of impregnated agar. In slide tests, French bean rust, *Uromyces appendiculatus*, was completely inhibited by $25\ \mu g\ ml^{-1}$ sclareol (8.3×10^{-5} M) and its germ tubes were very much shortened at $5\ \mu g\ ml^{-1}$. In the presence of sclareol, broad bean rust (*U. viciae-fabae*) produced small protuberances at the surface of the uredospores and normal growth of the germ tube occurred only at levels below $25\ \mu g\ ml^{-1}$. The results with *Puccinia recondita* f. sp. *tritici* (wheat leaf rust) were more variable, but again normal growth of the germ tube occurred only if the concentration of sclareol was below $25\ \mu g\ ml^{-1}$ (Table 1). Similar results were obtained using impregnated agar.

Protective fungicidal effects were measured by spraying sclareol, dissolved in a 50% v/v acetone–water mixture, on to leaves to the run-off point before inoculation[2]. In replicate experiments, applications of $100\ \mu g\ ml^{-1}$ sclareol consistently gave good control of rust on French bean, broad bean and wheat, reducing infection to less than 10% of control. Thus sclareol, a natural component from the leaves of *N. glutinosa,* can be used to protect other plants from disease.

Table 1 Effect of sclareol on the germination and growth of rust uredospores

Concentration of sclareol (μg ml^{-1})	Test no.	*U. appendiculatus*	*U. viciae-fabae*	*P. recondita*
5	1	35 (20)	100 (100)	76 (100)
	2	32 (33)	100 (100)	80 (100)
10	1	28 (20)	94 (100)	45 (60)
	2	23 (33)	100 (50)	60 (100)
25	1	0 (0)	81 (8‡)	36 (20)
	2	0 (0)	26 (25)	16 (100)
50	1	0 (0)	0 (†)	37 (20)
	2	0 (0)	0 (25)	20 (100)
100	1	0 (0)	0 (†)	23 (10)
	2	0 (0)	0 (†)	16 (60)
250	1	0 (0)	0 (†)	13 (20)
	2	0 (0)	0 (†)	16 (60)
500	1	0 (0)	0 (†)	16 (10)
	2	0 (0)	0 (0)	23 (60)
1,000	1	0 (0)	0 (†)	27 (10)
	2	0 (0)	0 (0)	27 (60)
	1*	60 (400 μm)	96 (480 μm)	92 (400 μm)
	2*	94 (240 μm)	62 (160 μm)	75 (400 μm)

Figures are the germination and (in parentheses) mean germ tube length obtained in two separate slide tests, both values expressed as percentage control.

*In the absence of sclareol, the percentage germination and length (μm) of the germ tube were as shown.

†Small protuberances present.

‡Germ tubes swollen and distorted.

In subsequent experiments sclareol levels up to 500 μg ml^{-1} failed to control powdery mildew on cucumber or wheat, anthracnose on cucumber or chocolate spot on broad bean. It is clear that as a protectant, sclareol is highly specific for rust fungi and that its capacity to control fungal disease is closely correlated with the effects it produces *in vitro*. Thus an examination of the surface of broad bean leaves, which had been sprayed with 100 μg ml^{-1} sclareol and inoculated with rust, showed that, whereas germination of the rust spores was almost completely inhibited, spores of epiphytic fungi were germinating normally. This specific toxicity towards rust fungi may arise as an extreme expression of the suppression of hyphal extension noted with other fungi[1], since rust fungi growing *in vitro* or on the leaf surface are normally devoid of lateral hyphal branches[3].

Little is known about the physiological mechanisms controlling fungal growth or about the endogenous compounds which may regulate such growth processes as hyphal branching, appressorium formation or haustorium formation. This situation contrasts markedly with knowledge of higher plant growth regulation—fully exploited for the development of selective hormone-type herbicides and of growth retardants, which interfere with the action of plant growth regulating hormones[4].

The present results, however, indicate that a study of endogenous fungal growth regulators and of compounds, such as sclareol and griseofulvin[5], which may mimic their action, offers an approach that could prove rewarding in research on the selective control of plant disease.

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Inhibition of pulmonary tumour development in rats sensitised to rat embryonic tissue

PREVIOUS investigations have established the presence of tumour-associated embryonic antigen(s) at the cell surface of virus-induced, chemically-induced and spontaneously arising tumours[1–4]. *In vitro* tests have shown that rat tumour cells, expressing the embryonic components, act as appropriate targets in cytotoxicity assays using lymph node cells from animals sensitised to embryonic tissue[3,5] (L.P.S., R.C.R. and R.W.B., unpublished). In these tests lymph node cells, from either multiparous rats or rats immunised against embryonic tissue, either by inoculation of irradiated 14–15-d-old rat embryo cells or embryonic excision, were shown to be cytotoxic for a large range of experimental rat tumours. Translation of these studies to the *in vivo* situation has not been uniformly successful in all the tumour systems studied. Some investigators have shown that embryo immunisation can induce immunity to subcutaneous challenge with minimal doses of tumour cells[6,7]. Conversely other laboratories, using similar immunisation procedures and tumour models have been unable to confirm these observations (refs 8–10 and L.P.S., R.C.R. and R.W.B., unpublished). It has previously been established that intravenous inoculation of rat tumour cells leads to the development of pulmonary tumour growth[11,12]. Studies were therefore initiated to investigate whether or not presensitisation of rats to rat embryonic tissue could prevent the growth of tumours in the lungs. The results presented here show positive reductions in pulmonary tumours in rats sensitised to embryonic tissue compared with untreated controls.

The tumours used in the present study have previously been shown to produce lung nodules following intravenous inoculation of single tumour cell suspensions[11,12]. Epithelioma Sp1 arose spontaneously in a female rat from our own inbred colony, and sarcoma Mc7 was induced by subcutaneous inoculation of 3-methylcholanthrene. The tumours were maintained by serial transplantation into rats of the same sex. Single tumour cell suspensions were prepared by trypsin digestion, washed in Hanks' BSS and resuspended in Hanks' BSS at a concentration of 5×10^6 cells ml^{-1}.

Groups of rats were immunised with three weekly inoculations of irradiated (5,000 rad) 14–15-d-old rat embryo cells

Table 1 Incidence and number of pulmonary tumour nodules in Wistar rats sensitised against syngeneic rat embryonic tissue and challenged intravenously with a methylcholanthrene-induced rat sarcoma (Mc7) or a spontaneously arising epithelioma (Spl)

Test	Immunisation procedure	Tumour cells	No. of rats with pulmonary nodules	No. of pulmonary nodules	*P*
1	Nil	Sarcoma Mc7*	4/5	0, 7, 9, 9, 17	
	Irradiated rat embryo cells (×3)		2/4	0, 0, 1, 1	<0.02
2	Nil	Sarcoma Mc7*	3/4	0, 17, 181, 300+	
	Embryoma excision		1/4	0, 0, 0, 3	<0.04
3	Nil	Sarcoma Mc7†	4/4	7, 12, 22, 36	
	Multiparous female rats		2/5	0, 0, 0, 1, 1	<0.004
4	Nil	Epithelioma Spl*	4/5	0, 2, 7, 8, 10	
	Irradiated rat embryo cells (×3)		5/5	1, 2, 2, 3, 4	<0.3
5	Nil	Epithelioma Spl†	4/4	7, 9, 11, 11	
	Multiparous female rats		1/4	0, 0, 0, 3	<0.004

*Rats challenged with 1×10^6 tumour cells (0.2 ml volume) intravenously 7–10 d following the third immunisation or after surgical excision of a developing embryoma.

†Multiparous female rats having had three or more litters were challenged intravenously with 1×10^6 tumour cells (0.2 ml volume) within 4 weeks following the last pregnancy.

(L.P.S., R.C.R. and R.W.B., unpublished). Rats were inoculated with 2×10^6 (0.2 ml volume) rat embryo cells subcutaneously and 1×10^6 cells (0.1 ml volume) intraperitoneally. Seven to ten days after the final inoculation both treated and control rats were inoculated intravenously with 1×10^6 tumour cells (0.2 ml volume). Multiparous rats having had three or more pregnancies, and rats grafted with 14–15-d-old rat embryo tissue and the embryoma excised when 1–2 cm in diameter, were similarly inoculated intravenously with sarcoma Mc7 or epithelioma Spl cells. Experiments were terminated 4 to 6 weeks after tumour cell inoculation and the lungs examined for pulmonary tumours as previously described[13]. The significance of the results was analysed by the Wilcoxon non-parametric rank test.

Using sarcoma Mc7 cells, significantly fewer tumour nodules developed in the lungs of rats immunised against 14–15-d-old rat embryo tissue, by either inoculations of irradiated rat embryo cells or by embryoma excision, compared with control rats (Table 1, experiments 1 and 2). Similarly, multiparous rats also developed significantly fewer lung tumours than nulliparous controls (Table 1, experiment 3). A reduction in lung tumours was also observed in rats sensitised to embryonic tissue and challenged intravenously with epithelioma Spl cells (Table 1, experiments 4 and 5), although these results were shown only to be statistically significant in experiments using multiparous rats challenged with Spl cells. In all experiments shown in Table 1 the size of lung nodules detected in embryo-immune rats was considerably less than those seen in control animals.

Previous studies from this laboratory have shown that inbred Wistar rats sensitised to embryonic tissue do not consistently reject a subcutaneous challenge with syngeneic tumour cells derived from either chemically-induced or spontaneously arising rat tumours (ref. 8 and L.P.S., R.C.R. and R.W.B., unpublished), and conflict with reports from other laboratories using similar tumour systems and immunisation procedures[7,14,15]. Lymph node cells and spleen cells prepared from multiparous pregnant rats or rats immunised with embryonic tissue do, however, demonstrate cytotoxicity *in vitro* for target 14–15-d-old rat embryo cells and tumour cells[5,16] (L.P.S., R.C.R. and R.W.B., unpublished). Various theories can be proposed as to why embryo-immune rats do not reject *in vivo* challenge with tumour cells expressing embryonic antigens at their cell surface, thus allowing immunological recognition and destruction *in vitro*. First, the nature of embryonic antigen expression may vary depending on conditions, and in the *in vivo* situation may assist in making the embryonic antigen component an inappropriate target for rejection to occur. Alternatively the nature of the immune response of the host to embryonic antigens may not be sufficient to facilitate a rejection response, and there is evidence to suggest that the cellular immune response to tumour-associated embryonic antigens is effected by a different immunological mechanism from that operative towards the tumour-specific component (J. Bray, R.C.R., R. A. Robins and R.W.B., unpublished).

The results presented here show that embryo-immune rats are capable of limiting the growth of tumours in the lungs. Thus, intravenous inoculation of tumour cells may facilitate direct contact with cytotoxic effector mechanisms early on, and so prevent the establishment of the tumour. On the other hand (or in addition), tumour cells may be unable to lodge in the lungs because of mechanisms other than those of an immunological nature. Studies are in progress to determine whether inhibition of pulmonary tumour growth is caused by a specific immunological response to the antigens present on 14–15-d-old rat embryo and rat tumour cells.

We thank Mrs M. E. Marshall and Miss J. McVeagh for assistance. This work was supported by a grant from the Cancer Research Campaign.

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Received March 14; accepted April 14, 1975.

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Stationary and dynamic responses during visual edge fixation by walking insects

If the visual surround of a walking animal is rotated, the animal turns in a characteristic way. With arthropods, this optomotor (optokinetic) response has usually been described in terms of the angular velocity of the body or eyestalks. The difference between these velocities—the slip speed—has been considered as the necessary visual stimulus. Most of the previous investigations of this feedback system have been carried out in open loop conditions[1]. Special care was taken that the head of the tethered animal was not free to move. In this investigation, free moving animals with head movements unrestricted also show optomotor responses when no slip speed between animal and surround is present. An explanation is offered which is in accordance with investigations on the housefly, *Musca domestica*[2,3].

In the experiments the mealworm beetle, *Tenebrio molitor*, walks freely on the vertex of a dark grey sphere (80 mm in diameter) which is servo-controlled to eliminate displacement (but not rotation) of the beetle with respect to the vertical axis of a surrounding rotatable cylinder (100 mm high and 94 mm in diameter), carrying the visual panorama on its inner surface. The animal carries on the thorax a small disk of highly reflecting foil, which is imaged in infrared light on the centre of a photodiode device with independent quadrants. The photocurrents control two servomotors, which rotate the ball to cancel translation of the beetle. Because the animal ordinarily walks with its body axis at a tangent to its track, one can derive two voltages, U_θ and U_v, proportional to angular direction θ and linear velocity v, respectively.

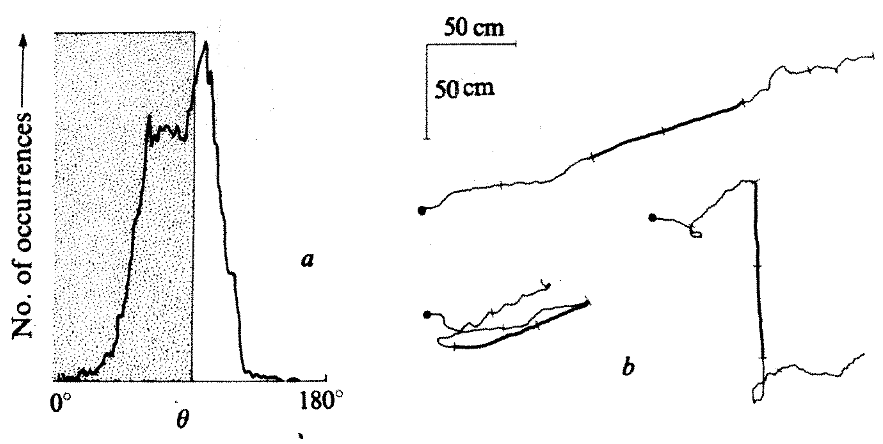

Fig. 1 *a*, Angular distribution of the direction of movement of a mealworm beetle during a 2 min period of walking, sampled every 5 ms. The shaded bar represents the black half of the visual panorama; the object of fixation is the vertical border separating it from the white half. *b*, Tracks of three beetles, in the dark (thin lines) and during illumination (heavy lines); a single black stripe subtending 22.5° in azimuth was presented in different angular positions. ●, Starting points; cross bars, 0.5 min time intervals.

Sixteen small light bulbs, arranged symmetrically above a screen of translucent white paper, provide an adequately uniform illumination of the visual panorama, which subtends an angular altitude from 26° below to 59° above the horizontal plane of the animal.

Figure 1 illustrates the walking behaviour measured with stationary panoramas. In Fig. 1*a* the response edge fixation is shown: a histogram of samples of the direction of movement over a 2 min period (U_θ sampled every 5 ms). Here the panorama was a half-white, half-black surround; on average, the beetle walked towards the right edge of the black field.

Figure 1*b* demonstrates that the walking direction is visually guided. Here the panorama was a single black stripe subtending 22.5° in azimuth. The tracks of the beetle are reconstructed by integrating the voltages proportional to the velocity components v_x and v_y and plotting them against one another on an x–y recorder. The beetle ran for 1 min in the dark (thin lines); the lights were then turned on for 1 min (heavy lines) and finally off again for a further minute (thin lines).

In the dark, even though the track is somewhat tortuous, a certain average direction is maintained. On illumination, direction alters immediately towards that of the black stripe and the track becomes straighter. If the dark direction happens to correspond to that of the stripe, as in the upper track, direction is unchanged. Surprisingly, the initial direction in the dark is resumed when the lights are turned off. Although it has not been excluded that this behaviour depends on a clue within the experimental arrangement, similar phenomena have been observed with the millipede, *Schizophyllum sabulosum*[4].

When the drum is rotated, the beetle tries to maintain fixation of the black stripe. At moderate angular velocities it succeeds (Fig. 2*a*). The average angular velocity of the animal during this dynamic fixation is identical to the angular velocity of the drum. With increasing drum velocities, however, the animal lags more and more behind the stripe, suddenly makes a fast turn against the direction of the drum's movement, locks on to the next stripe behind and fixates again for a while (Fig. 2*b*). The duration of these fixation periods becomes shorter and shorter as the angular velocity of the drum is further increased.

If the panorama consists of several black and white stripes of equla width, at moderate drum velocities the beetle fixates one of the black stripes for long periods of time; occasionally it switches to a different stripe. Usually the change is to a stripe following the one previously fixated, but in some cases (Fig. 2*c*) the beetle overtakes the drum; there it switched from black stripe No. 1 to black stripe No. 3. The net result of these small but frequent backward jumps is that the average angular velocity of the animal is less than that of the drum.

Open loop investigations[1] suggest that the direction and the magnitude of the optomotor response should not change if the panorama is rotated with constant angular velocity. Furthermore, it has been concluded that the difference between the angular velocities of the panorama and the animal is the necessary stimulus for the response; the two velocities should never[5], or only at their lowest values[6], be equal. The image of the surround would therefore be expected to be continuously displaced with respect to the retina.

My findings do not confirm these expectations, but the contradiction can probably be resolved. Fixing the head of the tethered animal to the thorax allows the precise control of the relative angular velocity between the eyes and the panorama but also prevents scanning movements of the head. It has been shown, however, that in stationary illumination conditions in the housefly, at least a random relative movement—instead of an unidirectional slip speed—between the eyes and the panorama is both necessary and sufficient to elicit fixation reactions such as those shown in Fig. 1. Since such scanning movements were not suppressed in the present investigations, it is suggested that the turning response of the animal shown in Fig. 2*a–c* can be accounted for as fixation of moving edges rather than as velocity compensation.

Equation (1) has been adapted from Reichardt[2] to describe fixation response:

$$\mathrm{d}^2\theta/\mathrm{d}t^2 + a(\mathrm{d}\theta/\mathrm{d}t) = ky(\gamma - \theta) \qquad (1)$$

where γ is the angular position of a fixated edge, θ the direction of the body axis and a and k are constants. The function y, which depends only on the deviation $\delta = \gamma - \theta$ between the angular direction of the fixated edge and that of the body axis of the animal, describes the attractive force an edge exerts on the animal. Since this function has not yet been determined quantitatively for *Tenebrio*, it was arbitrarily chosen as shown in the inset of Fig. 2*d*. Analog computer solutions of equation

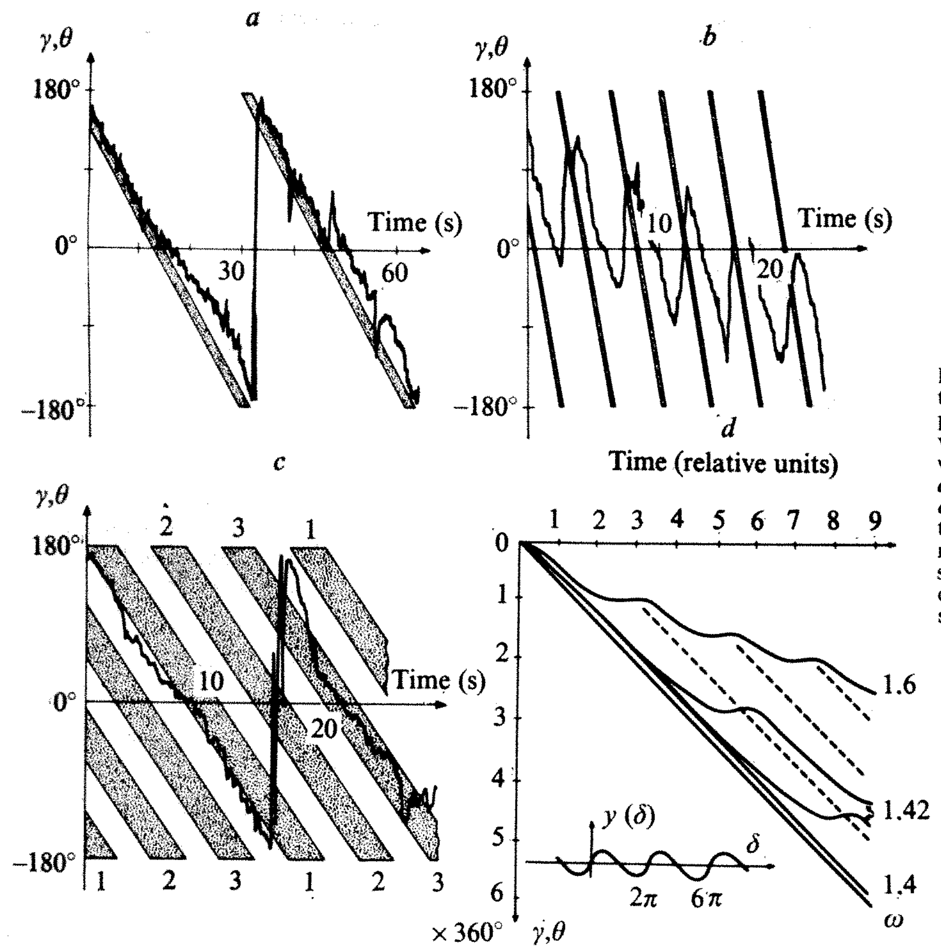

Fig. 2 *a–c*, Dynamic fixation of rotating patterns. The angular position γ of the rotating pattern (counter-clockwise, constant angular velocity) and the angular position θ of the walking beetle are shown against time. *a* and *b*, One 22.5° black stripe as in Fig. 1*b*; *c*, three 60° stripes. Angular values outside the range ± π rad are shifted by $n2\pi$ to the range of the plots. *d*, Analog computer simulation of the fixation response as described in the text. The waveforms for successive cycles have not been shifted to a single range of 2π rad.

(1) for $\gamma = \omega t$, where ω is the angular velocity of a single fixated edge, are shown in Fig. 2*d*.

As in my experiments, the angular position θ of the body axis deviates at moderate velocities ($\omega = 1.4$ relative units), lagging by a small constant amount behind the angular position γ of the fixated edge, whereas the two angular velocities are identical. At a critical value (here, $\omega = 1.42$) occasional jumps, and at still higher values ($\omega = 1.6$) frequent and regular jumps, of magnitude $\theta = 2\pi$ occur; each jump is followed by a period of dynamic fixation. The frequency of occurrence of such jumps, at a given drum velocity, depends on both $\gamma(\delta)$ and the parameters of equation (1). As a result of computer noise the jumps near the critical value of ω occur at irregular intervals. If the panorama contains more than one edge, the jumps occur at the respective neighbouring edge in less than 2π steps, as also observed in my experiments.

The qualitative properties of the theoretically derived curves agree well with those of the beetle's response. This does not exclude the possibility that the optokinetic reaction is important in the turning response of the animal. At higher drum velocities or increased number of stripes (see Fig. 2*b* and *c*) the backward jumps give rise to an unidirectional slip speed on the average. This in turn can elicit an optokinetic reaction in the sense of a velocity compensation, which is added to the fixation response.

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Received January 28; accepted April 11, 1975.

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Effect of experimental cerebral infarction in rat brain on catecholamines and behaviour

ALTHOUGH cerebral vascular accident or stroke is a frequent clinical problem, there has been little systematic research into the cause of the common behavioural consequences such as emotional lability, depression and motor and cognitive deficits. Ischaemic brain injury affects the content and metabolism of brain catecholamines in rats[1], monkeys and gerbils[2], as well as the concentration of monoamines in human cerebrospinal fluid[3]. In an attempt to study experimental stroke, we have occluded surgically the middle cerebral artery in rats and found, during the 40-d postoperative period, changes in both behaviour and brain catecholamines.

Sprague–Dawley male rats (150–200 g) were housed individually in a 12 h light and 12 h dark environment and given rat chow and water *ad libitum*. In one group of 30 rats, the right middle cerebral artery was ligatured with 6–0 suture just above the rhinal fissure, and severed distal to the ligature. In a second group of 25 sham-operated rats the artery was exposed, but not ligatured. The ligature procedure causes no postoperative mortality and the lesion always destroys only cortical tissue in the lateral aspect of the fronto-parietal cortex, above the rhinal fissure and far lateral to the cingulum bundle. The lesion extends through the entire depth of the cortex in most animals but in a few affects only the upper 60–75%. Even when the lesion extends through the entire depth of the cortex, however, there is no histological or fluorescence histochemical evidence of subcortical tissue damage. From above, the lesion

Table 1 Catecholamine concentrations after vascular lesion

	Days after vascular lesion	5	20	40	5	20	40
		NA concentration			DA concentration		
Lesioned parietal cortex	Concentration	276±29	425±39	447±46	153±39	192±30	224±57
	% Change	−36%†	−28%‡	−24%*	0%	+25%	+7%
Contralateral parietal cortex	Concentration	356±31	484±21	560±28	138±32	192±51	239±74
	% Change	−17%	−18%	−5%	−10%	+25%	+14%
Ipsilateral posterior cortex	Concentration	182±19	340±47	368±55	41±6	131±33	94±8
	% Change	−45%‡	−30%*	−24%	−59%*	+30%	−43%
Contralateral posterior cortex	Concentration	233±40	425±18	417±43	55±9	125±16	139±46
	% Change	−29%	−13%	−14%	−46%	+24%	−17%
Ipsilateral brainstem	Concentration	720±28	782±58	784±70	260±25	260±44	129±13
	% Change	−34%§	−29%‡	−29%§	−31%†	−31%	−53%‡
Contralateral brainstem	Concentration	775±24	1055±45	1091±62	307±40	327±20	233±36
	% Change	−29%*	−4%	−1%	−19%	−14%	−14%
No. lesioned		10	9	7	10	9	7
No. sham-lesioned		6	7	7	8	7	7

Catecholamine (CA) concentrations are expressed as ng g^{-1} wet tissue weight (± s.e.m.). Below each CA value is the percentage change as compared with sham-operated controls. Changes in control CA levels seemed to be the result of normal progressive development of monoamine innervation in these animals which were 45-d-old at the beginning of the study (29, 30). The NA changes were: 429±31 to 589±32 in parietal cortex; 330±26 to 486±38 in posterior cortex and 1,084±70 to 1,098±54 in brainstem, all changes occurred between 5 and 20 d after sham operation. The DA changes were: 154±15 to 210±35 in parietal cortex; 101±13 to 166±32 in posterior cortex; and 379±22 to 272±39 in brainstem, all changes occurred between 20 and 40 d after sham operation.

*$P<0.05$, †$P<0.01$, ‡$P<0.005$, §$P<0.001$, catecholamine concentrations were compared with those of sham-operated controls, using Student's t test.

is circular and varies from 2 to 5 mm in diameter. Its location also varies depending on the distribution of the middle cerebral artery and the site of ligature (Fig. 1; inset). After the surgical procedure, one group of rats was subjected to performance tests while a second group was monitored for food intake, At 5, 20 and 40 d after the operation, groups of rats were killed and their brains were removed, dissected immediately and frozen for assay.

Six brain regions were analysed by a modification[4] of the radioenzyme assay[5] that determines picogram amounts of both noradrenaline (NA) and dopamine (DA) in tissue samples as small as 2 mg wet weight. Table 1 shows that in each region there was a unique pattern of change. In the cortex, the greatest deficits in NA occurred 5 d after operation; at 20 and 40 d NA levels approached or reached control values. This was also true of contralateral brain stem which was the only contralateral region to change significantly. The NA in the ipsilateral brain stem, however, remained 30% below control levels throughout the postoperative period. Brain DA did not change significantly except in the ipsilaterial brain stem where concentrations decreased progressively, and in the posterior ipsilateral cortex where concentrations had decreased by 5 d after operation.

We measured spontaneous horizontal and vertical activity in a photocell chamber during 24 h, after 1 h of habituation. The average hourly spontaneous horizontal activity of the lesioned rats was significantly greater than that of controls from 2 to 20 d after the operation (Fig. 1). In contrast, spontaneous vertical acivity of the two groups did not differ significantly throughout this period. Open-field observations revealed a left-sided forepaw weakness and decreased response to touch and pain for several hours after vascular occlusion. By 24 h, there were no grossly detectable motor or sensory deficits. Within each group, rats were paired before operation and tested in a standard shock box for shock-induced aggression[6]. Two independent observers rated responses either aggressive or non-aggressive (inter-rater correlation $r=0.96$, $P<0.001$). The shock threshold required to elicit vocalisation did not change after operation. The percentage of aggressive responses was greater in lesioned animals 2 d after operation compared with controls; however, from 15 to 30 d after the operation the percentage of aggressive responses in the lesioned rats was well below control levels (Fig. 1). Daily measurement of the amount of rat chow eaten (minus crumb spillage) and of body weight revealed no significant differences between lesioned, sham-operated or unoperated control animals (Fig. 1).

The fact that the pattern of behavioural changes after vascular lesion differed for each behaviour studied, suggests that occlusion of the middle cerebral artery affects only certain behaviours and does not have a generalised nonspecific action. Although these lesioned rats may have some motor-sensory deficit not detectable by open-field observation or response to touch, there is evidence that the behavioural changes were not entirely attributable to such deficits. First, non-stereotyped hyperactivity is scarcely what one would expect from a hemiparetic or hemianalgesic animal. Second, an animal with bucco-lingual paresis or impaired taste or smell would not be expected to eat normally. Finally, the response to shock induced aggression was biphasic in the lesioned animals while there was no change in their shock threshold for vocalisation.

The nucleus locus coeruleus is the cellular origin for cortical NA fibres which project to wide area of the cortex including a component to the contralateral side (refs 7–9 and R. Freedman, S. Foote and F.E.B. in preparation). The origin of the widespread DA cortical fibres[10,11] has not been determined. Electrolytic lesions of catecholaminergic fibres in the central nervous system result in changes of catecholamine syntheses or storage capacity in cell bodies proximal to the lesion[12,13], in distal terminal sites[14,16], and in regions remote from the lesion[17]. Thus, the initial decrease in catecholamine could result from release of local neuronal catecholamines following interruption of fibre pathways leading from brain stem to cortex.

The return of catecholamines to control levels by 20 or 40 d after operation may result from several mechanisms. For example, the changes in NA and DA we observed in the brainstem after cortical injury could represent altered metabolism which the perikarya of catecholaminergic neurones would be expected to show as the result of a shift in synthesis from catecholamines to material for axonal sprouting[17,20,24]. Alternatively, the return to control levels in cortical regions after lesioning could represent increased formation of catecholamines in existing terminals. Finally, the failure of catecholamine in some areas to return to control levels may represent some arrested development of neurones. Quantitative recovery of the behavioural indices, occurring over the same time, may similarly be the result of any one of several neural pro-

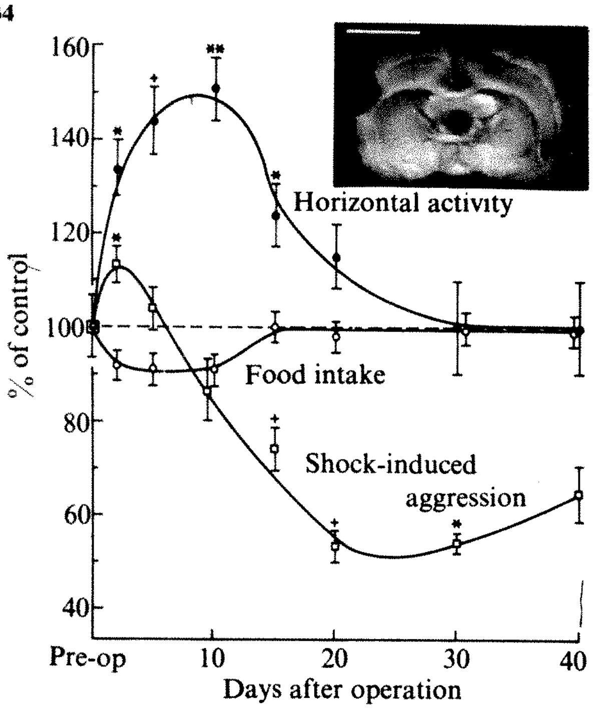

Fig. 1 Behavioural measurements after occlusion of right middle cerebral artery expressed as percentage of control. Spontaneous horizontal activity (●) is expressed as average hourly activity over 24 h. Preoperative level for both lesioned and sham-operated animals was 134 counts per hour. Chamber size was 40 × 32 × 35 cm with photocells 3 cm apart. Food intake (○) was measured in grams of standard dry rat chow consumed over 24 h with *ad libitum* feeding. Preoperative values for all animals were 22.4 g d^{-1}. Shock-induced aggression (□) was determined by subtracting the number of preoperative aggressive responses from the postoperative level. The change in the number of aggressive responses for a pair of lesioned animals was then compared with the change in the number of aggressive responses for a pair of sham-operated controls of the same postoperative interval. Shock given was 2 mA, r.m.s., scrambled shock, 0.5 s duration, 10 s intershock interval, total of 50 shocks. Bars indicate s.e.m. All values were compared with appropriate sham-operated controls using Student's t test; * $P<0.05$; + $P<0.01$; ** $P<0.001$. Inset: coronal cross section of rat brain 40 d after ligature of right middle cerebral artery. Note that lesion involves only the lateral aspect of the parietal cortex with no injury to the underlying caudate. Bar indicates 5 mm.

cesses. By 20 d after lesioning, histochemical fluorescence reveals a return of cortical fluorescence and evidence of growth of the spinous process normally found on somata in the locus coeruleus (ref. 25 and R. G. R., F. E. B. and E. Battenberg, in preparation). This supports speculation that the response of catecholamine neurones to injury may influence the pattern of biochemical and behavioural changes after operation. Stroke is associated with emotional changes in many patients[26,27]. In fact, epidemiological data suggest that stroke may play a role in the onset of endogenous depression in 10% of elderly patients[28]. Furthermore, decreased catecholamine concentrations have been implicated in the aetiology of numerous abnormal emotional changes including endogenous depression[31,32]. Although there are difficulties in drawing conclusions about humans based on animal experiments, we, nevertheless, believe this study provides evidence that the response of catecholamines neurones to vascular injury may cause the emotional changes seen after stroke and suggests the applicability of pharmacological treatments for these changes.

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Received December 11, 1974; revised March 24, 1975.

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Axonal degeneration associated with a defective blood–brain barrier in cerebral implants

The capillaries in the central nervous system (CNS) are unique with respect to their barrier mechanisms, preventing to a varying degree many types of substances from passing into the brain parenchyma from the blood. Such barriers exist also against the catecholamines and their immediate precursor, L-dopa[1–3]. As the blood–brain barriers serve to protect the central nervous elements against harmful substances in the circulation, it can be postulated that damage to or defects in the barriers could lead not only to functional disturbances but perhaps also to direct structural damage in the brain tissue.

We have found that cerebral implants of peripheral tissue become efficiently reinnervated by regenerating sprouts from lesioned central noradrenergic neurones[4–6]. Although the regenerated central fibres form functional connections in the transplant[5], however, and although the transplants survive well in part, the central fibres in many transplants later develop fluorescence histochemical signs of damage, resulting in a progressive terminal degeneration of sometimes the entire terminal network[6]. In contrast, such changes were not noted in the intracerebral portions of the same neurones.

We now report that these degenerative phenomena are associated with a failure of the blood–brain barrier to develop in the new circulation of the cerebral implant, suggesting that the central axons are insufficiently protected in the transplanted peripheral tissue. Our study has focused on the development of two of the characteristic barrier properties in the newly-forming capillaries: the enzymic trapping mechanism for L-dopa, and the barrier for catecholamines. For comparison the same problem was investigated in transplants of CNS tissue to the anterior chamber of the eye, a situation in which the CNS tissue is revascularised from peripheral vessels.

Sixty female Sprague–Dawley rats (170–200 g body weight at the time of surgery) were used. Two types of transplantations were made in aseptic conditions and using barbiturate anaesthesia (Brietal, Lilly, 40 mg kg^{-1} intraperitoneally): (1) autologous transplantations of irises to the caudal diencephalon, as described previously[4,6] (these animals were killed at 3, 5, 14, 30, and 60 d after operation (three to six animals in each group)); (2) homologous transplantations of embryonic CNS tissue to the anterior chamber of the eye (small pieces (about 1 mm^3) of the

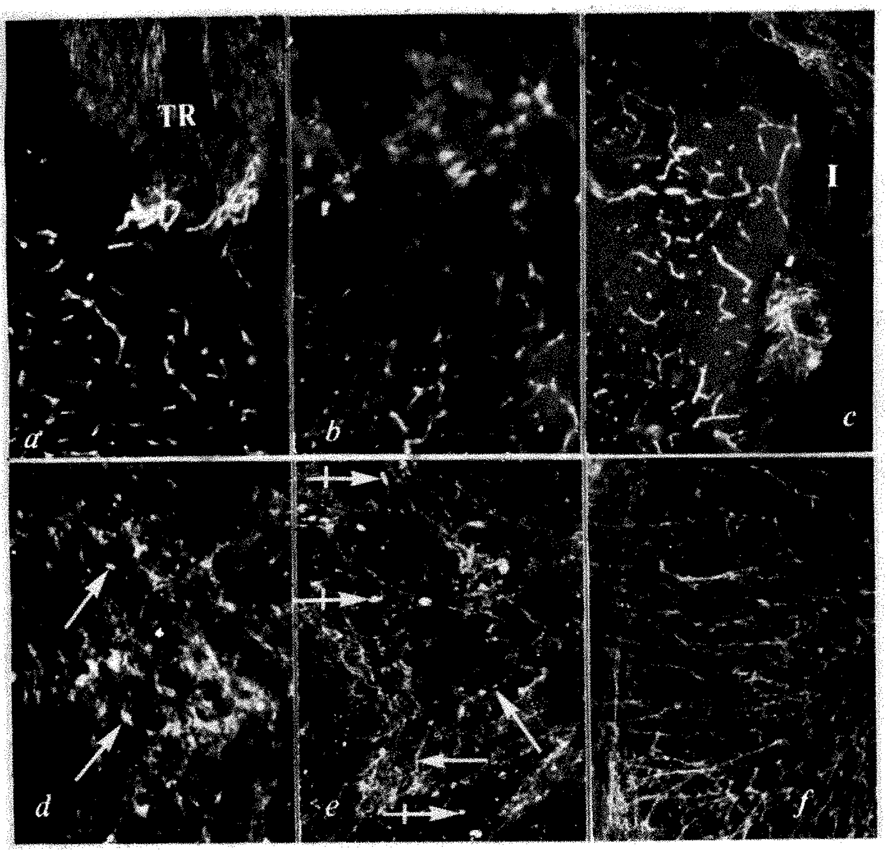

Fig. 1*a*, Ventral part of a 1-month-old iris transplant (TR) to the caudal diencephalon (× 88). The animal was treated with nialamide and L-dopa before being killed (see text). At the zone of transition between the ventral tip of the transplant and the parenchyma containing normally fluorescent capillaries, ample intensely fluorescent tortuous capillaries occur, none of which retains its fluorescence when entering the transplant. *b* and *c*, Embryonic CNS tissue from the locus coeruleus region transplanted to the anterior eye chamber (× 88). Nialamide and L-dopa treatment as in (*a*). *b*, 10 d survival. Normal or almost normal fluorescent capillaries are seen in the parenchyma. Fluorescent catecholamine cell bodies are seen in a cluster near the top of the picture. *c*, 30 d survival. Strongly fluorescent capillaries are seen all over the parenchyma up to the zone of transition between the CNS tissue and the iris (I), supplied with fluorescent noradrenergic sympathetic fibres. *d* and *e*, Comparison between disintegrating regenerated central noradrenergic fibres in whole mount diencephalic iris implants. *d*, Transplant from an animal treated intravenously with 6-OHDA (75 mg kg^{-1}, three injections at day 7, 14, and 21; 29 d survival (× 100). Site of ingrowth in the dilator of fibres showing characteristic signs of axonal damage. The fibres are distorted and swollen and several distended axon stumps are seen (arrows). *e*, Regenerated central terminal noradrenergic plexus in the dilator under spontaneous resorption in a 2-month-old diencephalic iris implant (× 113). Arrows show axons in various stages of disintegration, and crossed arrows denote distended axon stumps. *f*, Whole mount iris transplant from caudal diencephalon (1 month survival; × 113) Ciliary region to the left in the picture. Adjacent dilator area, comparable to that in (*e*), shows a principally normal noradrenergic ground plexus without signs of degeneration.

brain stem were taken from embryos with a crown–rump length of 15–30 mm, and placed in the anterior eye chamber of the adult recipient rats[7,8]; these animals were killed after 5, 10, and 30 d (five to ten animals in each group)).

All transplanted animals, except those treated with 6-hydroxydopamine (6-OHDA), were given nialamide (Niamid, Pfizer, 100 mg kg^{-1} intraperitoneally) and 1 h later L-dopa (50 mg kg^{-1} intraperitoneally), and were killed by decapitation 20 min after the last injection. From the brains, the iris transplants were either processed as whole mounts (3–14 d specimens), or dissected out together with surrounding brain tissue (30–60-d specimens) and freeze-dried. From the eyes, the irises with attached CNS transplants were dissected out and freeze-dried. Fifteen of the animals bearing ocular CNS implants were perfused with Indian ink by way of the ascending aorta immediately before being killed. All specimens were processed for fluorescence histochemistry according to the Falck–Hillarp formaldehyde method[9].

In one set of experiments (10 animals) the ability of 6-OHDA to pass into the cerebral iris implants was tested. Half of them were given a single injection of 75 mg kg^{-1} 6-OHDA intravenously 1 month after transplantation and killed 3 d later. The others were given three injections of 6-OHDA (75 mg kg^{-1} intravenously) 7, 15, and 21 d after surgery, and were killed on day 29. The iris transplants were processed as whole mounts for fluorescence microscopy as above. A further two animals were perfused at 6 weeks after transplantation with trypan blue (10 ml by way of the ascending aorta) immediately before being killed.

As described previously[1,2], the systemically injected L-dopa is trapped in the capillary walls of the normal brain tissue, and shows in the fluorescence microscope an intense greenish fluorescence in the endothelial cells and the pericytes. *In vivo* and *in vitro* experiments[2,3] have shown that the cellular fluorescence primarily represents dopamine, formed locally from the exogenous L-dopa by decarboxylation. Inhibition of monoamine oxidase results in a strong accumulation of dopamine, which is reflected in the intense fluorescence in the capillary wall cells. This phenomenon—which is unique for capillaries in the CNS—represents a barrier mechanism for L-dopa, and it rests on two special properties of the wall cells of the cerebral capillaries: first, the presence in the cells of a decarboxylating enzyme that efficiently converts L-dopa to dopamine; and secondly, the relative impermeability of the endothelial cells to catecholamines[2,3].

The irises implanted in the cerebral tissue are rapidly revascularised, both from the brain parenchyma and the pial vessels. As shown in an earlier study[6], using perfusion of the vascular system with a fluorescent plastic particle suspension, a circulation in the transplant starts as soon as 1 d after transplantation. At day 5 a sparse network of vessels supplies almost the entire transplanted tissue, and at 3 weeks the vascular density is close to that of the normal iris.

The capillaries in the implanted iris were tested for their barrier properties towards L-dopa at times varying between 3 and 60 d after transplantation. No fluorescent capillaries were detected within the iris tissue in any of the experimental groups. Figure 1*a* illustrates normally fluorescent capillaries in the lower portion of the picture. At the zone of transition between the parenchyma and the transplant (that is, within the scar tissue surrounding the transplant) an abundance of intensely fluorescent tortuous capillaries occur, whereas none may be seen inside the transplant. When, in the microscope, switching between ultraviolet light and phase-contrast optics we could observe directly how the fluorescent capillaries in the border zone lost their fluorescence abruptly as they penetrated into the iris tissue.

The lack of a normal blood–brain barrier for amines in the implanted irises was further substantiated through intravenous injections of 6-OHDA. This neurotoxic catecholamine analogue does not normally pass through the blood–brain barrier. The ability of systemically administered 6-OHDA to cause damage to catecholamine axons in the CNS has therefore been used as an index for the efficiency of the blood–brain barrier[10]. In animals bearing iris transplants in the CNS, the 6-OHDA treatment resulted in a total destruction of the regenerated noradrenergic terminal network in the iris. The axons had degenerated up to the site of ingrowth from the brain tissue (Fig. 1*d*), that is, up to the border zone between the transplant and the surrounding brain parenchyma. The fluorescence microscopical picture of the lesioned axons resembled that observed in the regenerated central noradrenergic terminal network in 2–4-month-old iris transplants of animals not treated with 6-OHDA (Fig. 1*e*). For comparison a corresponding part of the dilator from a 1-month-old transplant is shown in Fig. 1*f*. At this stage the regenerated central adrenergic terminal network shows no sign of spontaneous axonal degeneration.

In the reverse experiment—transplanting embryonic CNS tissue to the anterior chamber of the eye—the central tissue is revascularised from the peripheral vessels in the host iris[6,11]. By as early as 5 d after transplantation—a time when the revascularisation of ocular implants has been found to be almost complete[6]—the wall cells of the capillaries within the CNS transplant showed a moderate fluorescence after L-dopa treatment. A strong capillary fluorescence was found 10 d and 30 d after transplantation (Fig. 1*b* and *c*). Fluorescence was never seen in the capillaries of the host iris or in the vessels bridging between the host iris and the CNS transplant. That the fluorescent capillaries in the CNS tissue were functioning was shown in the specimens in which the vascular system had been perfused with Indian ink. In these specimens Indian ink was found in the lumen of the fluorescent capillaries.

Our results thus show that in the new circulation established from the cerebral vessels in the transplanted peripheral tissue, the capillaries will not acquire the normal barrier properties towards amines and amine precursors, characteristic for cerebral capillaries. In the animals perfused with trypan blue there was an extravasation of the dye confined to the iris transplant, suggesting that there may be a more general defect in the capillaries of cerebral implant. In the reverse situation, with ocular implants of CNS tissue, the new circulation established from peripheral vessels in the cerebral tissue exhibited a fluorescent microscopically visible blood–brain barrier towards exogenous L-dopa, and these properties developed as soon as the new circulation was established. These observations show that the barrier properties of the new circulation are determined not by its origin, but by the revascularised tissue.

One possible explanation for this is that the new vascular supply occurs solely in the original capillary network of the transplants. For several reasons this seems unlikely. Thus, it has been found in skin grafts[12,13] that a recirculation in parts of the original vascular bed will occur during the first few days after transplantation. From the day 4 to 12, however, these vessels degenerate and, concomitantly, there is an ingrowth of new vessels into the graft from the transplantation bed[12]. From our own study of the revascularisation process of irises implanted in the caudal diencephalon[6] it also seems that a recirculation of original vessels might occur in some restricted areas of the transplant during the first few days. From the second postoperative week onwards, when the new vascularisation extends within the iris, the vascular supply changes, however, into such irregular and abnormal patterns that may only be explained in terms of the formation of a new capillary network.

There is thus very good support for the assumption that the capillary networks at least in the cerebral implants are newly-formed, and consequently, that the barrier characteristics of the newly-formed capillaries are determined by the properties of the tissue in which the new circulation occurs. There seems to be two principally different mechanisms whereby this could be established, either through the recruitment of old or dormant endothelial cells from the transplanted tissue for the building of the new capillaries, or—if the capillaries grow in from the transplantation bed—the barrier properties would be actively induced from elements in the cerebral tissue. It is interesting that after a mechanical or electrolytic lesion in the brain there is a pronounced regeneration of the cerebral capillaries into the lesion, and—in contrast to those forming in the peripheral transplants—these newly-formed vessels acquire the barrier properties for L-dopa soon after they are formed[14]. It seems conceivable that the same regeneration mechanism could operate in the revascularisation of both transplant and scar tissue and that the development of a blood–brain barrier in the wall cells is dependent on the tissue into which the capillaries grow.

From the present observations it is evident that the cerebral iris transplant, reinnervated by regenerated central noradrenergic fibres, provides an interesting situation in which central axons extend out from the barrier-protected central tissue into the unprotected environment of the transplant. Our previous studies on this reinnervation process[4-6] have shown that the central fibres survive well during the first few weeks (Fig. 1*f*), and they establish within a month after transplantation functional contacts with the smooth muscle in the transplant[5]. During the second and third postoperative months, however, the central fibres in the transplant often show signs of direct structural damage, and at 2–4 months after transplantation the central terminal network has in many cases degenerated (Fig. 1*e*).

The blood–brain barriers are generally regarded as mechanisms whereby harmful substances in the circulation are excluded from entering the CNS. In situations where the barrier function is damaged or deficient—such as in the cerebral iris transplant—the CNS elements should therefore be more vulnerable. In a region of the CNS normally lacking blood–brain barriers, namely the pituitary gland, the central innervation shows in fact abundant ultrastructural and fluorescence histochemical signs of acute damage, and it has been suggested that the axons innervating this unprotected region undergo continuous degeneration–restitution cycles [15,16].

The integrity of the blood–brain barriers therefore seem to be necessary for the protection of cerebral tissue from direct structural damage. It has been proposed[17,18] that abortive regeneration in the CNS may be attributable to an inhibition of axonal regrowth by immunological mechanisms that become mobilised and obtain access to the lesioned axons because of the breakdown of the blood–brain barriers at the site of injury. According to this theory the unprotected brain sites will expose brain autoantigens, normally sequestered by the blood–brain barriers, and sensitised cells or autoantibodies will attack the site of lesion at which they have free access to the antigens[18]. Disturbed vascular permeability in the CNS has also been implied in other pathological conditions, for example, in multiple sclerosis[19,20]. If, indeed, a deficient blood–brain barrier is the cause of long term degeneration of central axons, this could be a primary aetiological factor in neurological disorders.

This work was supported by grants from the Swedish MRC.

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Received March 12; accepted April 4, 1975.

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Contractility of smooth muscle cells of rabbit aorta in tissue culture

THE involvement of smooth muscle cells (SMC) from the media of elastic arteries has been implied in the aetiology of atherosclerosis[2]. Aortic tissue cultures have been studied repeatedly to examine the ability of SMC to synthetise macromolecular proteins, and collagen and elastic fibres. Ross[3] has shown that aortic cultured cells retained all the morphological characteristics of SMC up to week 8 of culture. By week 4 microfibrils identified as elastic fibres appeared close to the cells. On the other hand, Ouzilou *et al.*[4] showed that the cultured aortic fragments can be used as a suitable model to study the metabolic properties of aortic smooth muscle. The explants retained, for at least a week, the ability to synthesise the proteins of the medial matrix at a rate that decreased with the age of the rabbits from which the aortic specimens were obtained.

To correlate the synthetic activity with the physiological state of the SMC in tissue culture, we have examined the response to drugs and electrical stimulation of both the explants and cells at different stages of evolution. The contractile response of the explants and cultured cells from young rabbits was tested in a Zeiss inverted microscope with phase contrast optics enclosed in a temperature-regulated chamber at 37 °C. The movements were monitored and registered in a videotape recorder or filmed with an Arriflex camera at a speed of 2.5 frames per s. The films were later projected at 24 frames per s. Drugs were added to the Petri dishes filled with 5 ml Hanks' solution to obtain final concentrations of 10^{-5}M angiotensin II (At$_{\text{II}}$), 10^{-5}M noradrenaline (NA), 10^{-5}M 5-hydroxytryptamine (5 HT) and 50 mM KCl. On the other hand the explants and cells were stimulated electrically by glass electrodes filled with 3 M NaCl with a 1 MΩ tip resistance. Square-wave pulses had a maximum intensity of 10^{-5} A and 1 s duration.

No contractile response of subcultured cells, confluent for 3 weeks or non-confluent, was detected under the action of drugs and electrical stimulation in 30 experiments. This confirms a previous report by Martin and Sprague[6]. In one experiment only, confluent cells in a 21-d primary culture (Fig. 1) contracted with NA and KCl without response to At$_{\text{II}}$. An undulatory movement affected most cells in the fields. Groups of cells organised in parallel bundles contracted at the same time, asynchronously with respect to other contiguous bundles. This contraction shortened single cells very slightly and was very slow. To observe this, the film had to be projected at fast speed. The duration of each contraction was between 10 and 20 s. In contrast with the cultured cells, for up to 3 weeks the explants always responded with a contraction synchronous with the electrical stimulation.

To quantify the spasmogenic response of the aortic fragments, helical strips maintained in culture for different periods

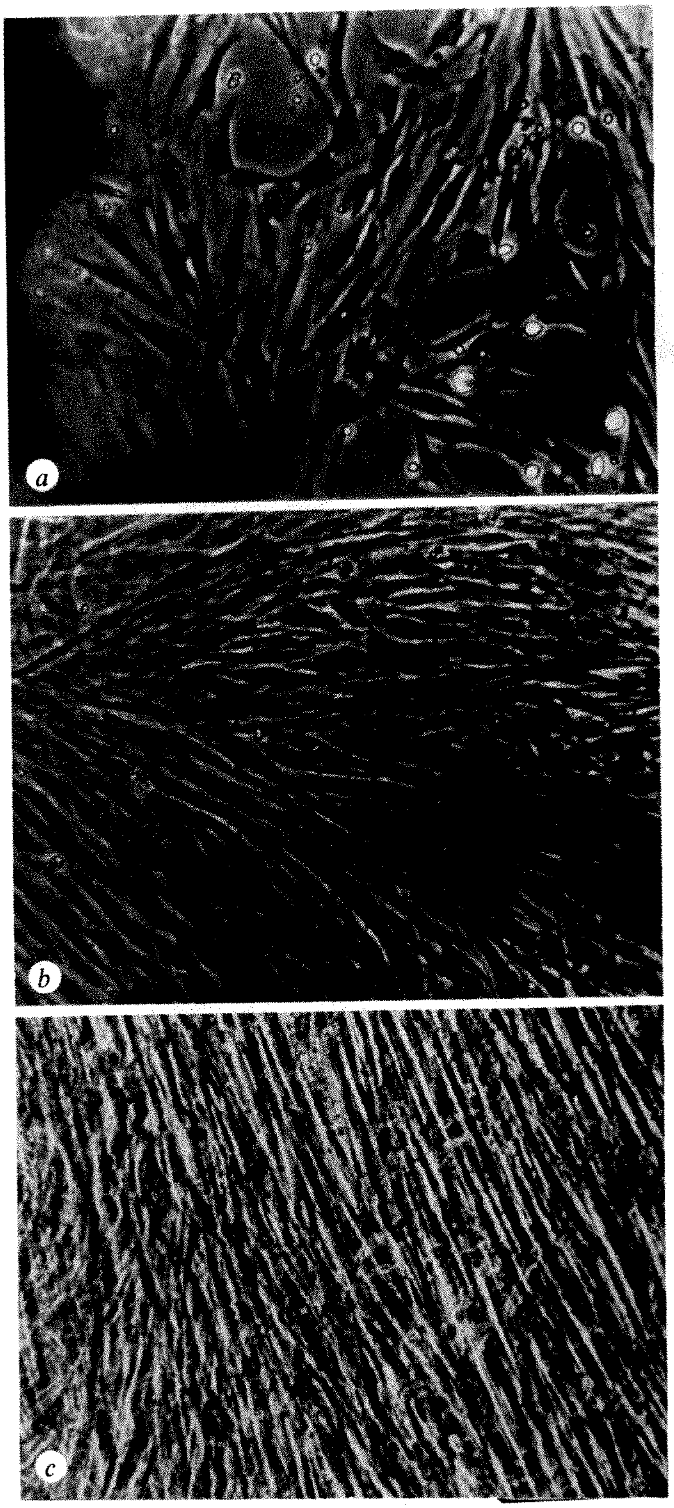

Fig. 1 *a*, Phase-contrast photography of spindle-shaped cells growing out of young rabbit aortic explants (dark area) after culture for 11 d (×145). These cells did not contract with electrical stimulation, 5-HT, NA, KCl or At$_{\text{II}}$. *b*, Phase-contrast photography of aortic cells at very high density. These cells have been subcultured once and kept confluent for 4 weeks. Criss-crossed multilayers can be observed. There was no contractile response with either drugs or electrical stimuli (×125). *c*, Photography from an area of a long term (22 d) primary culture which contracted with 10^{-5}M NA and 50 mM KCl. There was no response to 10^{-5}M At$_{\text{II}}$. Young rabbits of either sex (about 800 g) were killed by a blow on the head. The thoracic aorta was excised, washed in Hanks' solution, and the adipose tissue and adventitia removed, at room temperature. Fragments of about 1 mm^2 were put in Petri dishes in MEM Eagle's solution in Earle's buffer, to which 10% calf serum (GIBCO) had been added. Five or six fragments were installed in each dish under glass slides and equilibrated with a 95% O$_2$ and 5% CO$_2$ mixture. The media were renewed twice a week. In some cases helical strips have been prepared by cutting the aortae at an angle of 20° (ref. 5). Strips were then kept in Petri dishes and cultured in the same media as the smaller fragments, but kept free (without slides) in a gyratory shaker at 10 r.p.m.

were installed successively in 10 ml organ baths, regulated at 35 °C and perfused with modified Ringer solution[7]. The isotonic contractions of the strips attached to writing levers that provided a fourfold magnification at a constant tension of 2 g, were recorded on smoked drums. Cumulative dose–response curves to NA and At_{II} were obtained by exposing the strips to increasing concentrations of the drugs in the chamber once the contraction provoked by the previous dose had been stabilised. The strips were washed between doses, when the spasmogenic action of KCl was tested. Typical experiments are presented in Fig. 2. Very rapidly, after 1 d in culture conditions, the reactivity to NA started to decrease, and disappeared completely at day 3, when the response to At_{II} started to decline, and was practically suppressed at day 8, when the strips still contracted when depolarised by KCl.

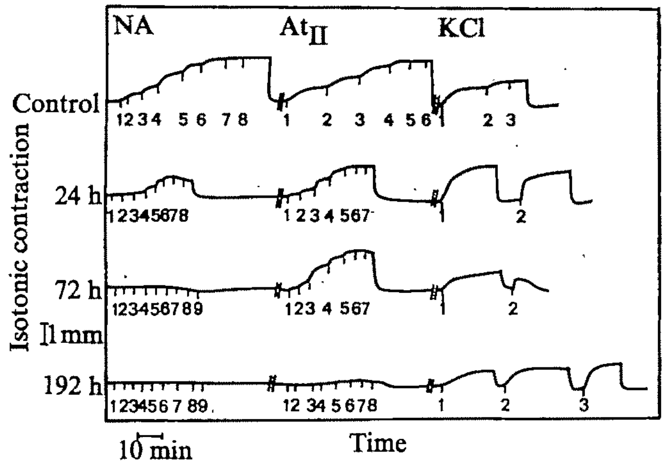

Fig. 2 Evolution of the contractile response of aortic strips after 24, 72 and 192 h culture. Cumulative dose–response curves to NA and At_{II}, and non-cumulative curves in the presence of a solution in which NaCl is partially replaced by KCl. Drug concentrations were: NA(M): 1, 1×10^{-8}; 2, 2×10^{-8}; 3, 5×10^{-8}; 4, 2×10^{-7}; 5, 5×10^{-7}; 6, 2×10^{-6}; 7, 5×10^{-6}; 8, 2×10^{-5}; 9, 5×10^{-5}; At_{II}(M): 1, 2×10^{-9}; 2, 5×10^{-9}; 3, 2×10^{-8}; 4, 5×10^{-8}; 4, 2×10^{-7}; 6, 5×10^{-7}; 7, 1×10^{-6}; 8, 2×10^{-6}; KCl (mM K^+): solutions containing 1, 34; 2, 67; 3, 101.

It is interesting to compare these results with previous studies concerned mainly with the morphology of aortic cultured SMC. Cells in primary cultures, which grow out from the explants and are characterised by a lower myofilament content and an increase in organelles (defined by Fritz *et al.*[8] as modified SMC), never contract under the action of drugs and electrical stimulation. Subcultured confluent cells, which are currently used as an experimental model for atherosclerosis, are nearly always non-responsive. This lack of spasmogenic response does not seem to be related to an artefact of culture, as in the same experimental conditions isolated cells of guinea pig taenia coli and vas deferens contract spontaneously and when stimulated with carbamylcholine or NA (J.P.M. and M.W., unpublished). Cells of primary cultures may recover the functional capacities of normal aortic SMC as shown by the fact that we were able once to observe a contractile response to NA and depolarisation.

Explants keep their contractility for long periods. Coincidently the normal histological structure of the organ is conserved during week 1. During week 3, the muscular layer is disrupted but some SMC are still arranged in organised bundles in some loci, mostly in the periphery of the explant, as shown by Fritz *et al.*[8]. As there is no response to At_{II} or NA at this time, however, it must be admitted that either the drug receptors disappeared or there is a fault in the excitation or the excitation–contraction coupling mechanisms. This problem was further explored with the dose–response curves of aortic strips. In fact there is a difference in the timing of the decrease in the response to drugs, the spasmogenic effects of NA being suppressed before those of At_{II}. This suggests *per se* that their receptors are modified or their number decreases, besides any other change in cell excitability or excitation–contraction coupling. From our results and other histological studies, we can conclude that aortic SMC dedifferentiate when they grow out of the explant. Their cell membrane properties are modified during this process. This phenomenon is slower in the explant probably because of matrix–cell interaction. Subcultured confluent cells are not similar to normal *in vivo* SMC as far as their contractile properties are concerned.

To confirm whether proliferative SMC *in vitro* can be used as a model for the study of atherosclerosis, it will be necessary to determine the physiological status of the cells that proliferate in atheromatose plaques to find out whether SMC lose their contractility in these conditions.

This work was supported by grants from the CNRS and INSERM. We thank K. Robert, M. Moczar and P. Meyer for support and criticism.

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Received February 25; accepted April 11, 1975.

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Allosteric inhibition of the sodium pump by external sodium

WHEN the sodium pump works forward performing Na–K exchange, it is activated by intracellular Na and extracellular K (refs 1–3), but is also inhibited by internal K and external Na (Na_0) (refs 4–8). So far, the inhibition by internal K seems adequately described in human erythrocytes, as fully competitive at three internal sodium sites[7,8], but the situation regarding the Na inhibition at the external K sites seems more complicated. The experiments reported here suggest an allosteric interaction of external Na with those sites.

In the presence of Na_0, the curves showing the activation of the pump by external K are sigmoid, while in Na-free medium the curve is apparently hyperbolic; the $K_{\frac{1}{2}}$ for K is lower in Na-free medium, whereas the maximal activation is the same as that in high-Na medium[5,6,9,10]. The kinetics of activation of the pump in red cells by external K in the presence of high Na_0, has been interpreted assuming that two K ions must bind externally for Na–K exchange to occur[11], probably with similar affinities. Even in nominally Na-free medium, the activation of ouabain-sensitive K influx by K_0 has been found to be slightly sigmoid at very low K_0 concentrations[6], and for similar conditions, Lew *et al.*[12] have proposed a two-site activation of Na efflux by external K, with a large difference in dissociation constants (4 and 100 µM).

When the ouabain-sensitive K influx in red cells is measured at fixed K_0 concentrations and variable external Na, Dixon plots ($1/v$ against Na_0 concentration) show a linear competitive inhibition by Na_0 (refs 10 and 13). This would imply that whereas two K ions are needed for the external activation of the pump, one Na ion is enough for inhibition. The linear dependence of $1/v$ on Na_0 has been confirmed for cells loaded to have a high internal Na concentration, prepared by the pCMBS method[14]. Similar results for nystatin-loaded cells[15] are presented in Fig. 1*a*.

The linear inhibition by Na_0 has been interpreted as dead-end at only one of the K sites[14]. If it were at the low affinity site, this could only lead to a pseudo-Michaelian behaviour, that is, the activation of ouabain-sensitive K influx by K_0 should be hyperbolic at all Na_0 concentrations; this situation is clearly not met in practice[3,6,10,11]. Two alternatives remain which are compatible with the observed results: (1) dead-end inhibition at the high-affinity site and (2) binding of Na_0 at neither of the K sites but at a third (allosteric) site (the 'partially-competitive inhibition' case of Dixon and Webb[16]). Binding of Na_0 at a third site only, should lead to a reversible change of the state of the K-binding sites, decreasing their affinities differentially to make them seem nearly equivalent at high Na_0 concentrations. This alternative implies that (1) the effect of the 'allosteric transition' is only on the K-affinity and not on the maximal velocity (compatible with the 'direct interaction by vicinity' of Monod *et al.*[17]) and hence the complexes KXK and $\overset{\text{Na}}{\text{KXK}}$ should lead to the same rate of K translocation inwards and (2) the affinity of the inhibitory site for Na_0 is lower when both K sites are loaded than when no K or only one K is bound[16].

A convenient test between allosteric as against dead-end inhibition is provided by the inspection of Dixon plots[18] of ouabain-sensitive K influx against Na_0 concentration, at very low concentrations of K_0. In the case of dead-end inhibition, the Dixon plots will still be linear; whereas in the case of allosteric inhibition, they will bend downwards and approach a hyperbola[18] since in this case there is a limiting non-zero K influx in the presence of saturating Na_0 concentrations. One of several experiments, which gave closely similar results, is shown in Fig. 1*b*. The inhibition of K influx is clearly a non-linear function of Na_0 in these conditions. If the inhibition were hyperbolic, it should conform to the general equation

$$1/v=(1/v_{i=0}-1/V_{\mathrm{max}})\,[(1+[\mathrm{Na}_0]/\mathrm{K}_{\mathrm{Na\ num}})/ /(1+[\mathrm{Na}_0]/\mathrm{K}_{\mathrm{Na\ denom}})]+1/V_{\mathrm{max}} \qquad (1)$$

(Compare Dixon and Webb[16]), formally analogous to those described by Cleland[19]. If the value of the ordinate for zero-Na_0 is subtracted from each curve we obtain

$$1/v-1/v_{i=0} = (1/v_{i=0}-1/V_{\mathrm{max}})([\mathrm{Na}_0]/\mathrm{K}_{\mathrm{Na\ num}}-[\mathrm{Na}_0]/ /\mathrm{K}_{\mathrm{Na\ denom}})/(1+[\mathrm{Na}_0]/\mathrm{K}_{\mathrm{Na\ denom}}) \qquad (2)$$

In general, and for any number of K sites

$$1/v-1/v_{i=0} = A/(1+\mathrm{K}_{\mathrm{Na\ denom}}/[\mathrm{Na}_0]) \qquad (3)$$

where A is independent of Na_0 and represents the maximal value of the function. The conformity with equation 3 of each set of data in Fig. 1*b* was tested by plotting the reciprocal of $(1/v-1/v_{i=0})$ against the reciprocal Na_0 concentration[19]. Good straight lines were obtained by least squares regression (linear regression coefficients > 0.96), from which the following parameters were derived (mean ± s.d.): (1) 0.027 mM K_0; $A = 157 \pm 39$ mmol^{-1} × l cells × h, $K_{\mathrm{Na\ denom}} = 37.1 \pm 10.2$ mM and (2) 0.053 mM K_0; $A = 56.5 \pm 11.5$ mmol^{-1} × l cells × h, $K_{\mathrm{Na\ denom}} = 45.4 \pm 9.9$ mM. The curves drawn in Fig. 1*b* represent the hyperbolae calculated from the above parameters. The values of A imply that at 0.027 mM K_0, the minimal K influx attainable at infinite Na_0 is 0.00613 mmol per l cells per h (96.9% inhibition of control influx), while at 0.053 mM K_0 it is 0.0172 mmol per l cells per h (96.7% inhibition). Both $K_{\mathrm{Na\ denom}}$ values are, however, not statistically different. This is in agreement with equations 2 and 3 which show that while A is a function of $1/v_{i=0}$, $K_{\mathrm{Na\ denom}}$ is an independent parameter; it represents the dissociation constant for Na binding at the third site, once the two K sites have been occupied[16].

To minimise changes in the external specific activity of ^{42}K resulting from leaking from the cells, all the experiments like that of Fig. 1*b* were conducted at the lowest feasible haematocrits (0.05–0.1%). The variation of the supernatant K concentration (mean of initial and final) between the zero-Na_0 and the highest-Na_0 tubes was usually of about +5%; an independent control experiment (not presented) showed that the

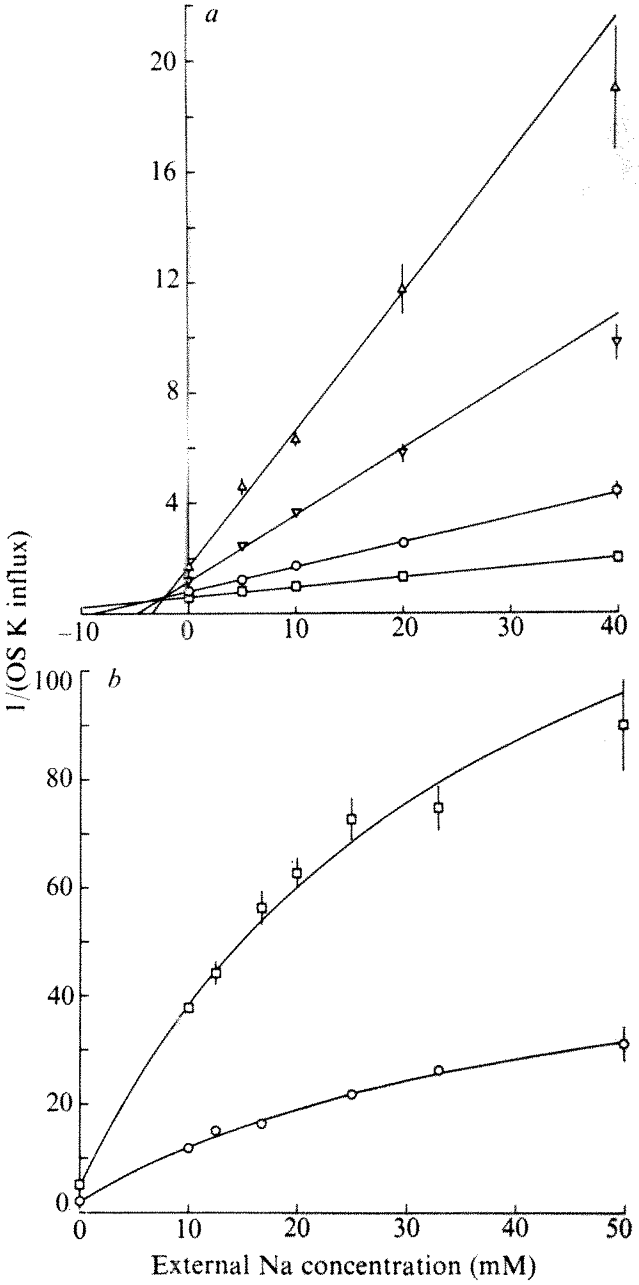

Fig. 1 (*a*) Linear inhibition of the ouabain-sensitive (OS) K influx by external Na. Red cells loaded by the nystatin method against: 80.5 mM Na, 52 mM K and 20 mM choline were used. Washed cation-loaded cells were resuspended in a cold medium containing: 130 mM choline, 2 mM Mg, 15 mM Tris (*p*H 7.9 at 20 °C) 153.5 mM chloride, 10 mM glucose. ^{42}K influx was then measured in triplicate at the external K and Na concentrations shown (by choline replacement) ± 10^{-4} M ouabain. The incubation was for 40 min at 37 °C, in a volume of 5.25 ml and at a final haematocrit of 0.34%. The bars indicate ±s.e.m. on the reciprocal velocities, calculated according to Wilkinson[24]. The lines have been drawn by eye. △, 0.077 mM K_0; ▽, 0.12 mM K_0; ○, 0.21 mM K_0; □, 0.35 mM K_0. *b*, Hyperbolic inhibition of the ouabain-sensitive K influx by external Na, at low external K. Erythrocytes loaded as in *a*. ^{42}K influx was measured in quadruplicate at 0.027 (□) and 0.053 (○) mM K_0 (average concentrations as assayed in the supernatants), at varying Na_0 concentrations (choline chloride replacement) ± 10^{-4} M ouabain. The incubation was for 60 min at 37° C, in a volume of 16.8 ml and at a final haematocrit of 0.08%. The reciprocal ouabain-sensitive K influx has been plotted against Na_0 concentration. The bars indicate ±s.e.m.; the lines represent the hyperbolae fitted as described in the text.

associated change in external specific activity at the highest-Na_0 with respect to the zero-Na_0 tubes could account for not more than −2% deviation of the highest point from a straight line drawn through the points at zero and 10 mM Na_0. On the other hand, the possibility that the observed behaviour of the K influx was caused by the existence of a small and constant component of the K influx due to K–K exchange, can be ruled out since the ouabain-sensitive K–K exchange in red cells is completely abolished at the internal Na and K concentrations used[20].

Having proposed a third external pump site which does not bind K, the question remains as to whether Na-binding to this site leads to Na translocation inwards. Since $K_{\text{Na denom}}$ is about 40 mM, in conditions of saturating external K concentration (say 15 mM K_0 at 130 mM Na_0), one would predict a relatively large ouabain-sensitive Na influx (25% of that at 0 K_0, 130 mM Na_0). This is clearly not the case, since Garrahan and Glynn[21] have shown that there is a negligible ouabain-sensitive Na influx in the present conditions.

But, the supposition that the allosteric site plus the two K sites constitute the three external Na sites for Na–Na exchange in the absence of external K, seems attractive[22]. The disagreement between this idea and the negligible ouabain-sensitive Na influx at saturating K_0 could be reconciled if only $\overset{\text{Na}}{\text{NaXNa}}$ (and possibly $\overset{\text{Na}}{\text{KXNa}}$) can lead to ouabain-sensitive Na influx, but not $\overset{\text{Na}}{\text{KXK}}$. In this connection, it will be recalled that when the ouabain-sensitive Na efflux from red cells is measured in a K-free medium and the Na_0 concentration is increased from 0 to 150 mM, the uncoupled Na efflux reaches a minimum at about 5 mM Na_0 (refs 12 and 23) before Na–Na exchange is turned on. Since the affinity of the third site for Na_0 should increase maximally in the absence of external K, the third external site is a likely candidate to be the one involved in the inhibition of the uncoupled Na efflux by low Na_0 concentrations.

Recent studies of ATP hydrolysis in resealed ghosts as a function of Na_0 lend support to this idea (I. M. Glynn, personal communication). If this were the case, binding of Na to the third site only (in the absence of K_0), would not lead to ouabain-sensitive Na influx, but would act instead as a 'brake' for the pump. The increase of Na_0 concentration beyond 5 mM (always in K-free medium) would then lead to occupation of the two K sites by Na and hence to Na–Na exchange. This implies that the affinity for Na_0 would be different at the different sites, although Garay and Garrahan[7] concluded that three identical low affinity sites existed for Na–Na exchange.

It is then possible, that the allosteric Na site responsible for the partially competitive inhibition of K influx, the high-affinity Na site responsible for the inhibition of the uncoupled Na efflux and the 'missing' site necessary for a three-site activation of the ouabain-sensitive Na influx, are just different states of the same binding group: a third external (Na) site of the Na pump.

We thank Drs I. M. Glynn and V. L. Lew for helpful discussion. J. D. C. acknowledges a fellowship from the Overseas Development Administration of the British Government, held at the time when this work was done.

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Molecular basis for formation of lipid sound lens in echolocating cetaceans

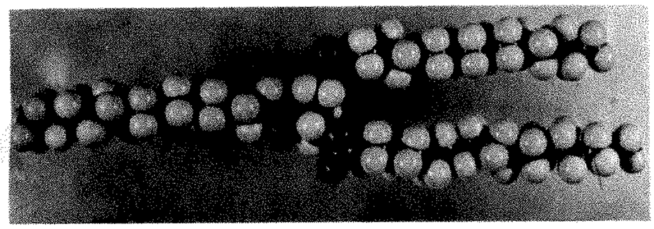
MOST echolocating porpoises and small whales (odontocete) possess a fatty protrusion on the forehead and a peculiarly formed lower jaw which flares into a posterior flange. Overlying this flange is a region of highly fatty tissue referred to by Norris[1] as an acoustic window. The fatty tissues (>80% lipid) of the mandible and the melon are believed to be associated with sound transmission[1-3]. It is proposed[4,5] that the melon functions as a sound lens, a concept consistent with the view that it is heterogeneous in terms of sound velocity and refraction[6]. Recent studies[7-13] revealed that the lipids of acoustic tissues are different from those of the surrounding tissues. For example, the acoustic tissues contain triacylglycerols and wax esters rich in short and medium chain (C_5–C_{12}) acids, whereas blubber tissues are composed primarily of triacylglycerols containing long chain (C_{11}–C_{22}) acids. In the families Delphinidae[7-9] and Monodontidae[12], isovaleric acid (3-methyl butyric acid) and longer iso (*gem*-dimethyl) acids are present in large amounts in acoustic tissues. Furthermore, certain areas, such as the acoustic window and the inner melon, contain almost exclusively 1,3-diisovaleroyl-2-acylglycerols and isovaleroyl wax esters (Fig. 1). These compounds are replaced by medium and long chain triacylglycerols in adjacent areas. Thus, specific lipid compositions with various proportions of branched structures are found in the putative areas of sound transmission[14,15]. The intriguing association between proposed acoustic pathways and unique lipid composition led us to study the acoustic properties of these lipids.

Our studies with acoustic tissues of the porpoise family Delphinidae relate lipid composition to ultrasonic velocity, density and compressibility. The results show that relatively small changes in the ratio of wax esters to triacylglycerols, both rich in branched structures, induce substantial alterations in the sound properties of the lipid medium. Moreover, we show here with a model system of trioctadecenoylglycerol (triolein) and hexadecyloctadecenoate (cetyl oleate) that these changes could not be achieved through the use of lipids commonly present in most animal tissues.

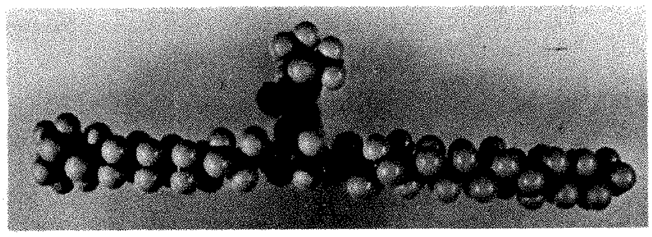
The velocity of ultrasound, c, is related to the compressibility and density by the following equation:

$$c^2 = \frac{1}{\beta d} \qquad (1)$$

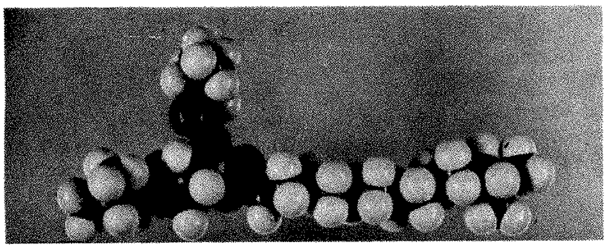
where d is the density of the medium and β is the compressibility. We measured the densities and ultrasonic velocities, and calculated the compressibilities of acoustic lipids pooled from several porpoises (*Stenella graffmani*). (The calculation yields the dynamic compressibility at the frequency of the ultrasound used in the velocity measurement (4 MHz). This value approximates the adiabatic compressibility. We can expect the values of velocity and compressibility to be valid at lower frequencies as well.) Pure triacylglycerols (65 g) and wax esters (15 g), which together comprise more than 99% of the acoustic lipids, were isolated by preparative thin-layer chromatography[7-10]. The



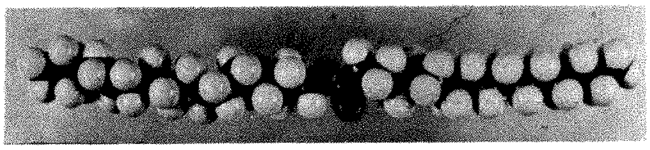
a **Long-chain triacylglycerol**



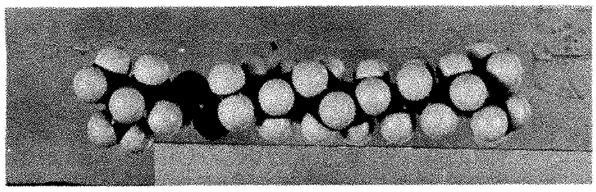
b **Monoisovaleroyldiacylglycerol**



c **1,3-Diisovaleroyl-2-acylglycerol**



d **Long-chain wax ester**



e **Isovaleroyl wax ester**

Fig. 1 Molecular models depicting several classes of triacylglycerols and wax esters present in porpoise acoustic tissues and other animal tissues. *a*, Long chain triacylglycerols containing long chain (C_{10}–C_{18}) acids on each carbon atom of the glycerol moiety are commonly found in animal tissues. In porpoise acoustic tissues, these triacylglycerols contain about 32 mol % of isoacids[13]. Specific example given here: 1,2-dipalmitoyl-3-stearoylglycerol. *b*, Monoisovaleroyldiacylglycerols, containing 2 mol of long chain acids for each mol of isovaleric acid, are present in small amounts in the porpoise acoustic tissues. Specific example given here: 1-isovaleroyl-2-isopentadecanoyl-3-palmitoylglycerol. *c*, 1,3-diisovaleroyl-2-acylglycerols, containing 2 mol of isovaleric acid for each mol of long chain acids, constitute a major fraction of the lipids of the acoustic tissues. These glycerides contain about 20 mol % of long chain isoacids[11]. Specific example given here: 1,2-diisovaleroyl-3-isopentadecanoylglycerol. *d*, Long chain wax esters, containing long chain (C_{10}–C_{18}) alcohols esterified with long chain acids, are commonly present in animal tissues. In the porpoise acoustic tissues, these wax esters contain about 40 mol % of isoacids[10]. Specific example given here: hexadecylhexadecanoate. *e*, Isovaleroyl wax esters, containing long chain alcohols esterified with isovaleric acid, constitute a significant fraction of the lipids from porpoise acoustic tissues. Specific example given here: isopentadecylisovalerate.

triacylglycerols contained 68% of 1,3-diisovaleroyl-2-acylglycerols, 11% of monoisovaleroyldiacylglycerols and 21% of long chain triacylglycerols (Fig. 1). (All percentages reported here are on a weight basis.) The wax esters contained 51% of isovaleroyl wax esters and 49% of long chain wax esters. Our measurements (Figs 2*a*, 3*a* and 4*a*) were limited to the mixtures of triacylglycerols and wax esters containing up to 30% wax esters, the maximum fraction present in most marine porpoises[14,15]. For comparison, measurements (Figs 2*b*, 3*b* and 4*b*) were made on mixtures of triolein and cetyl oleate (Nucheck Prep. Inc.). The ultrasonic velocity was measured with a conventional sing-around velocimeter at 4MHz. Because of the difficulty of purification of large quantities, only densities were measured for pure 1,3-diisovaleroyl-2-acylglycerols and wax esters (Fig. 2*c*).

The densities of all three sets of mixtures were less than the values calculated for ideal mixtures of liquids without intermolecular interactions (Fig. 2*a*, *b*, and *c*). The ultrasonic velocities of triolein–cetyl oleate mixtures were also lower than those calculated for ideal mixtures (Fig. 3*b*), although the deviation from the calculated values was not very large. In contrast, the lowering of the ultrasonic velocity was very pronounced for the triacylglycerol–wax ester system from the porpoise acoustic tissues (Fig. 3*a*). Curves of compressibility against composition are similar for porpoise triacylglycerol–wax ester and triolein–cetyl oleate (Fig. 4*a* and *b*). In the latter system (Figs 2*b*, 3*b* and 4*b*), however, the increase in compressibility is related

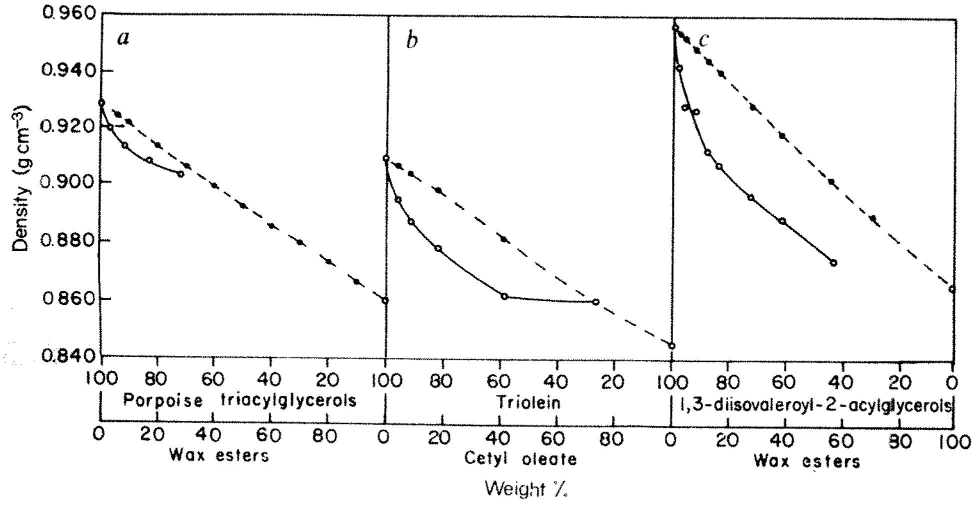


Fig. 2 Density against lipid composition. The measurements were made using a 2-ml pycnometer and an analytical balance. The samples were isolated in an atmosphere of nitrogen. Each fraction was dried carefully and traces of solvent were removed under high vacuum for 24 h at 40–50 °C. The measurements, made at 23 °C, had a precision of ± 0.003 g cm^{-3}. *a*, Porpoise triacylglycerols–wax esters system. Triacylglycerols and wax esters were obtained from acoustic tissues of several porpoises. *b*, Triolein (trioctadecenoylglycerol) - cetyl oleate (hexadecyloctadecenoate) system. *c*, 1,3-Diisovaleroyl-2-acyl-glycerols–wax esters system. Both fractions were obtained from the melon tissues of a single porpoise (*S. graffmani*). The wax esters, which contained 56 mol % isovaleric acid, exhibit a slightly higher density value than that of the wax esters in *a*. ●, Ideal mixture; ○, experimental.

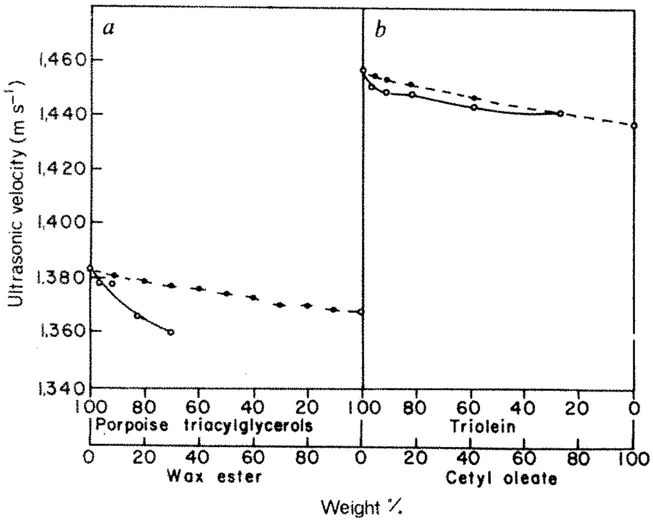
mainly to a decrease in density rather than in ultrasonic velocity, whereas in the former system, the increase in compressibility is related to a large decrease in ultrasonic velocity and a smaller decrease in density (see equation (1)). Thus the porpoise lipids have an ultrasonic velocity profile very different from that of the triolein–cetyl oleate system.

The ultrasonic velocity of porpoise triacylglycerols is much lower (1,383 $\mathrm{m\,s^{-1}}$) than that of triolein (1,460 $\mathrm{m\,s^{-1}}$) and decreases more rapidly as wax esters are added (Fig. 3*a*) than does the ultrasonic velocity of triolein on addition of cetyl oleate (Fig. 3*b*). Thus, by virtue of specific molecular structures and intermolecular associations of their components, porpoise acoustic lipids give rise to lower ultrasonic velocities which enable the required acoustic refraction to take place. Furthermore, the steep decline in ultrasonic velocity as a function of composition (Fig. 3*a*) allows the cetacean to control sound refraction by means of small changes in composition.

Norris and Harvey[16] have shown that in the melon of the porpoise, *Tursiops truncatus*, a central core of lipids of low ultrasonic velocity (1,273–1,376 $\mathrm{m\,s^{-1}}$) is surrounded by a graded high velocity shell (to 1,682 $\mathrm{m\,s^{-1}}$). Figure 3*a* shows that the porpoise triacylglycerol–wax ester system yields velocities of 1,360–1,383 $\mathrm{m\,s^{-1}}$ for compositions of 0–30% wax esters. It seems likely that the lower portion of the ultrasonic velocity range (1,273–1,360 $\mathrm{m\,s^{-1}}$) obtained by Norris and Harvey results from deposition of a virtually pure system of 1,3-diisovaleroyl-2-acylglycerols and wax esters because the acoustic window and the inner melon are composed almost exclusively of these compounds[14,15] and as our measurements of this system (Fig. 2*c*) show that the density declines steeply over a wide range of composition, a more pronounced decline than in either of the other systems.

The biochemical events that led to the deposition of the isovaleroyl lipids in areas of sound transmission can be explained as follows. At the site of deposition L-leucine provides the isovaleryl CoA that introduces isovaleric acid into the lipid structure[17]. Isovaleryl CoA also seems to undergo chain elongation to provide the longer chain isoacids[17]. In most mammalian systems isovaleryl CoA is degraded rapidly to acetate and acetoacetate[18]. Therefore, the modifications in biosynthesis that led to the formation of the lipids of acoustic tissues must have involved the activation of enzymes mediating the direct incorporation of isovaleryl CoA into lipid. Another advantage of depositing isovaleroyl lipids, which have the desired physical properties (low ultrasonic velocity, fluidity and stability to oxidation), is that their biosynthesis is largely independent of fluctuations in fatty acid distribution of dietary lipids[19].

Fig. 3 Ultrasonic velocity against lipid composition. The measurements were made at 23 °C, by a precalibrated sing-around velocimeter of 10 cm path length with a precision of ±1 $\mathrm{m\,s^{-1}}$. The temperature was measured with a calibrated mercury thermometer and is accurate to ± 0.5 °C. *a*, Porpoise triacylglycerols–wax esters system. The ultrasonic velocity value for pure wax esters was calculated using Schaaffs' equation[22] which relates molecular composition and density to ultrasonic velocity. *b*, Triolein–cetyl oleate system. ●, Ideal mixture; ○, experimental.



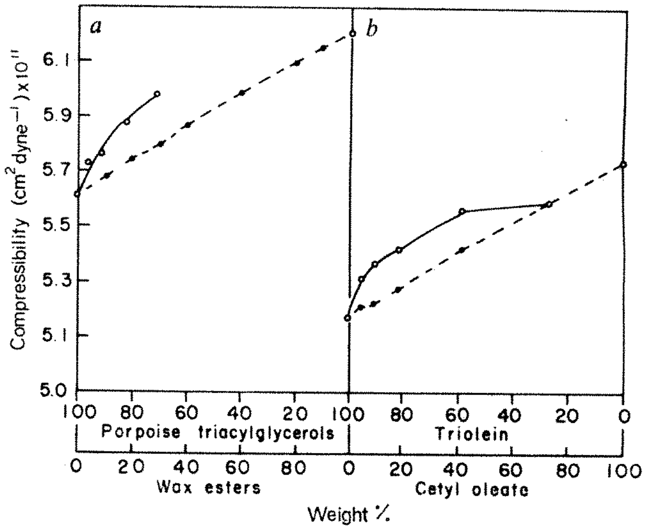


Fig. 4 Compressibility against lipid composition. Compressibilities were calculated from the measured values of density and ultrasonic velocity by use of equation (1). *a*, Porpoise triacylglycerols–wax esters system. *b*, Triolein–cetyl oleate system. ●, Ideal mixture; ○, experimental.

In summary, from our findings we can explain, on a molecular basis, why anomalous metabolic processes have evolved in echolocating cetaceans to form massive deposits of specialised lipids in certain areas of the head. It seems that one objective for the biosynthesis and deposition of acoustic lipids was the formation of a three-dimensional matrix of ultrasonic velocities that constitute a sound lens. The results show that the cetacean can react to changes in the environment by making a small change in the lipid composition, thereby achieving a major acoustic accommodation. We have suggested here how modifications in the metabolism of Delphinidae resulted in such an unusual and specialised role for lipid. On the basis of these results, we propose that the acoustic lipids containing short chain and medium chain acids found in other cetaceans[20,21] also serve in sound focusing.

We thank P. M. Falk and P. A. Robisch for assistance and W. F. Perrin, National Marine Fisheries Service, La Jolla for porpoise tissues. U.V. and D.C.M. thank the Biological and Medical Sciences Division, US Office of Naval Research, for financial support.

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Received February 6; revised April 2, 1975.

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Suppression of murine virus leukaemogenesis by thioglycollate, a bacteriological culture medium that affects macrophage peroxidase

INFECTION of mice with an oncornavirus such as Friend leukaemia virus (FLV) results in splenomegaly, hepatomegaly, blood cell dyscrasia and eventually death[1–3]. Such leukaemias can often be prevented or suppressed by either specific immunisation with a vaccine prepared from the virus or leukaemic cells[4–5], by treatment with non-specific enhancers of immune responsiveness such as bacterial lipopolysaccharides or polynucleotides[6–8], or by an interferon inducer like statalon[9,10]. Activated macrophages are thought to be the cells of the reticuloendothelial system primarily involved in nonspecific anti-tumour immunity. Since enhanced metabolic activity of phagocytes has been associated with wide spectrum microbicidal ability, it was interesting to determine whether thioglycollate treatment of mice would stimulate their macrophages and thereby affect FLV-induced leukaemogenesis. *In vitro* studies revealed that phagocytosis causes stimulation of peroxidase activity and H_2O_2 production by phagocytic cells from different sources[12]. It is well established that the combination of peroxidase, H_2O_2 and a halide such as chloride or iodide exerts a potent antimicrobial affect at acidic pH[13–16]. We now report marked inhibition of FLV-induced leukaemogenesis in mice injected with thioglycollate; this inhibition could possibly be related to increased peroxidase and glucose-6-phosphate dehydrogenase (G6PD) activities of peritoneal exudate (PE) cell macrophages in the treated animals.

Young adult ICR mice (Jackson Laboratories) were injected intraperitoneally with 0.5 ml of sterile thioglycollate medium (Difco). Two days later PE cells were collected; about 85% of them were typical macrophages. The peritoneal resident (PR) cells from untreated animals consisted of about 35% macrophages. PE and PR cells pooled from six to eight mice per group were tested for G6PD and peroxidase activities. G6PD was determined in intact cells suspended in glycerin by assessing NADPH formation as measured by the increased absorbance at 340 nm[17]. Peroxidase activity of cell homogenates was determined by guaiacol colour change at 470 nm[18]. The cell densities from both normal and treated mice were adjusted to 6×10^6 viable nucleated cells per cuvette. Table 1 shows that PR cells contain negligible amounts of peroxidase activity. PE cells from thioglycollate-treated animals contain significant quantities of this enzyme activity. PE cells also exhibit six times as much G6PD as PR cells.

Pretreatment with thioglycollate markedly suppressed FLV-induced leukaemogenesis (Fig. 1). An FLV preparation standardised by *in vitro* and *in vivo* assays for oncornavirus activity was used for these experiments[19]. Between 500 and 1,000 ID_{50} of a stock preparation of FLV, consisting of both leukaemogenic and focus-forming viral agents, free of LDH and LCM[19], were injected intraperitoneally into mice injected 2 d previously by the same route with thioglycollate. Spleen weights of these mice were significantly less than those of animals infected with FLV without previous treatment with thioglycollate (Fig. 1). Reduced spleen weights were evident in treated animals as late as 30 d after virus infection when control mice had spleen weights averaging 1,500–2,000 mg.

Mice infected with FLV generally show marked immunosuppression[20]. We, therefore, wanted to determine if thioglycollate could modify the effect of FLV infection on the immune response of mice to sheep red blood cells (SRBC). Additional groups of mice were injected intraperitoneally with 0.5 ml of thioglycollate 2 d before FLV infection. Eight days later these mice, as well as untreated controls, were challenged by interperitoneal inoculation of 0.5 ml of a 2% suspension of washed SRBC. Four days later the mice were killed and their spleen cells tested for haemolytic antibody plaque-forming cells (PFC) by the standard haemolytic plaque assay in agar gel[21]. Splenic PFC for control mice given neither virus nor thioglycollate were also determined. The results of a representative experiment (Table 2) show that mice injected with thioglycollate before FLV infection developed many more splenic PFCs than those given only FLV. Untreated FLV-infected animals responded to SRBC with fewer than 2,000 PFC per spleen. Control mice with SRBC alone had more than 68,000 PFC per spleen; uninfected mice given thioglycollate before SRBC developed about half this number of PFC. Infected mice pretreated with thioglycollate had more than 20,000 PFC per spleen; this was only moderately fewer than the PFC in the spleens of control animals injected with thioglycollate alone. The difference between the PFC response of FLV-infected mice pretreated with thioglycollate and that of untreated FLV-infected mice was highly significant ($P<0.001$).

G6PD is the initial enzyme of the hexose monophosphate shunt (HMS) in carbohydrate metabolism[22]. It is widely accepted that *in vitro* phagocytosis stimulates HMS activity and, therefore, H_2O_2 production[23–25]. Thus, the observed increased G6PD activity could indicate phagocytosis-associated HMS stimulation and increased H_2O_2 production.

Fig. 1 Comparative splenomegaly in mice infected with FLV, with or without intraperitoneal injection of sterile thioglycollate. Each point represents average spleen weight ± 1 s.e. for 12-15 mice on days 15 or 30 after infection with FLV (———) or for uninfected control mice (— — —), either untreated (○) or given thioglycollate (●) only.

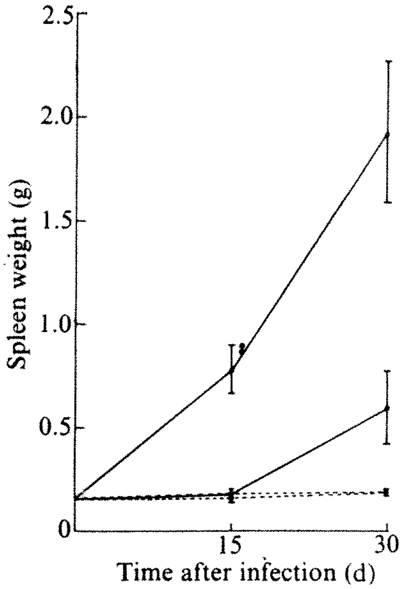

Table 1 Stimulation of glucose-6-PO$_4$ dehydrogenase and peroxidase activities in peritoneal exudate cells from mice treated with thioglycollate

Thioglycollate treatment*	Enzyme activity† G6PD	Peroxidase
−	0.239	<0.001
+	1.352	0.158

*PE cells pooled from either normal mice or mice injected intraperitoneally 48 h earlier with 0.5 ml of thioglycollate medium.
†Average activity for 6–8 determinations; G6PD activity expressed as μmol NADP reduced per min per 10^8 cells and peroxidase activity as guaiacol units per 10^8 cells.

It has been reported that activated macrophages have increased HMS activity[26] and are more phagocytic[27] than control macrophages.

The marked increase of peroxidase activity in thioglycollate-induced PE cells is similar to that reported by Simmons and Karnovsky[28] for casein-induced PE cells. From the results of our studies one could speculate that the peroxidase–H_2O_2–halide antimicrobial system can function *in vivo* and is effective against a tumorigenic virus such as FLV. Injection of thioglycollate has also been reported to induce plasminogen activator activity in PE cells[29].

We have noted a possible relationship between G6PD and peroxidase activities of thioglycollate-induced PE macrophages and suppression of FLV-leukaemogenesis. Data from preliminary experiments indicate that injection of thioglycollate up to 6 d after FLV infection significantly ($P<0.01$) reduces splenomegaly and that spleens from thioglycollate-treated FLV-infected mice contain less than 1% of virus assayed in untreated, infected mouse spleen cells. These results tend to show that thioglycollate treatment affects both the virus and newly transformed tumour cells.

Table 2 Suppressive effect of thioglycollate treatment of mice on FLV induced immunosuppression to SRBC

Mouse group*	Thioglycollate treatment†	Haemolytic PFC‡ Per spleen	Per 10^6 spleen cells
Control	−	68,350	441.7
	+	30,117	283.8
FLV-infected	−	1,359	10.4
	+	20,083	141.7

*Groups of mice, either normal or FLV infected, challenged intraperitoneally with 0.5 ml of 2% SRBC suspension.
†Treated mice given 0.5 ml of thioglycollate intraperitoneally.
‡Average number of PFC for 6–8 spleens for indicated mouse group 4 d after challenge immunisation with SRBC.

Activated macrophage peroxidase and G6PD activity could be involved in the observed inhibition of leukaemogenesis because the peroxidase–H_2O_2–halide system has been shown to have both virucidal[15] and anti-tumour effects[30–32] *in vitro*. It has been reported that thioglycollate-induced macrophages do not exhibit anti-tumour cell activity *in vitro*[33–35]. It has been shown, however, that mouse lymphoma cells exposed to 5×10^6M H_2O_2 for 60 min *in vitro* do not show changes in ^{51}Cr release, trypan blue uptake or 1-^{14}C-glucose oxidation but they no longer are able to induce tumours in susceptible mice[32]. Thus there may or may not be a relationship between *in vitro* and *in vivo* effects on a given parameter. Since the peroxidase–H_2O_2–halide antimicrobial system has been shown to have *in vitro* anti-tumour effects and injection of thioglycollate results in *in vivo* suppression of leukaemogenesis and elicitation of macrophages with increased peroxidase and G6PD levels, it seems that further studies of these and related metabolic activities could form the basis of a rational approach to prevention or therapy of some forms of malignancy.

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Received Januay 27, 1975.

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Melatonin may be synthesised in enterochromaffin cells

MELATONIN (*N*-acetyl-5-methoxytryptamine) is a hormone, originally isolated from the epiphysis of the bull[1], which is synthesised from 5-hydroxytryptamine (5-HT). Since the main site of synthesis of 5-HT is in the enterochromaffin cells of the intestinal tract[2] we investigated the possibility that melatonin is also formed there. We have found evidence in favour of this possibility.

Mucosa of human appendices was homogenised in saline and an alkaline extract was prepared as described previously[3]. The effect of the extract on frog skin melanophores was compared with the effects of 5-HT, melatonin, adrenaline and hydrocortisone. Each substance was administered into the lymphatic sac of frogs on five occasions, and in each substance we incubated pieces of skin taken from backs of ten normal frogs. Results were assessed by colour changes of skin[4,5] (50–60 melanophores in a microscope visual field magnified 56 times).

After incubation for 15–20 min, the mucosal extract produced changes in the frog's skin, consisting of an aggregation of pigment granules in the centre of cells around the nucleus, together with a change in shape of the melanophores, which assumed a compact, rounded form. There followed a gradual clarification of the melanophores, characteristic of the effect of melatonin. After 4–5 h the clarification became visible to the naked eye in as much as the outline of the pigmented areas became indistinct (Fig. 1). Solutions of adrenaline (0.1%), hydrocortisone (0.2%) and 5-HT (1%) did not clarify melanophores in the same period of time, although a solution of

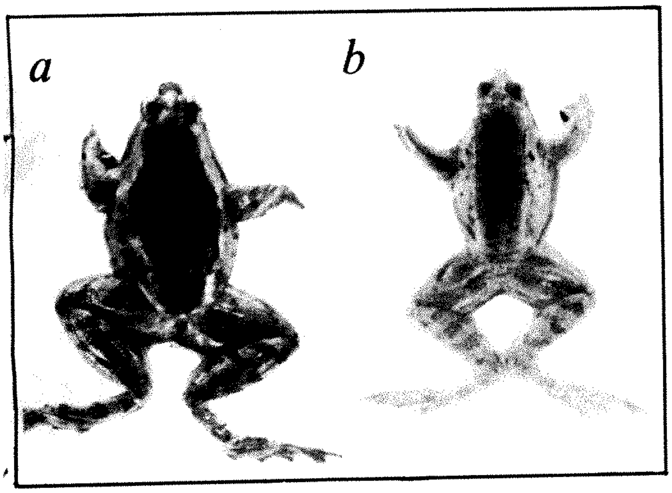

Fig. 1 Effect of alkaline extract of mucosa from human appendices on the melanophores of frog skin. *a*, Untreated skin; *b*, 5 h after administration of extract into the lymphatic sac.

melatonin (0.006%) in ethanol (30%) produced an effect comparable with the mucosal extract.

Thus an extract of the mucosal lining of human appendices contains a substance with a melatonin-like action. Since no direct demonstration of melatonin synthesis in human mucosa was produced, we investigated an intestinal extract of the rabbit, an animal known to contain large numbers of enterochromaffin cells. Thin-layer chromatography showed the presence of melatonin (R_f 0.73), together with 5-hydroxytryptophan (R_f 0.07), 5-HT (R_f 0.11) and 5-methoxytryptamine (R_f 0.32) (Fig. 2). Paper chromatography, using Filtrak F No. 1 paper with butyl alcohol–acetic acid–water, (12:3:5) or isopropyl alcohol–ammonia–water (8:1:1) as solvents, confirmed the thin-layer chromatography data, and also revealed traces of tryptamine (R_f 0.79 in the butyl alcohol-based solvent system).

The presence of melatonin precursors in the extract suggests that melatonin is synthesised in the intestinal mucosa rather than being transported there from some other site. Since the 5-HT is synthesised in the enterochromaffin cells[2] it seems likely that it is these cells which contain the melatonin and that it is synthesised there. Evidence to support this was obtained by a comparison of the melanophore-clarifying effects of mucosa, containing varying numbers of enterochromaffin cells, obtained from different cases of appendicitis (Table 1).

Our findings may be interpreted as supporting the suggestion[6] that the enterochromaffin cells, or the endocrine cells of the appendix which are producing melatonin, are derived from the neuroectoderm of the neural crest. The enterochromaffin cells belong to the amine precursor uptake and decarboxylation series of endocrine peptide-producing cells[7] which have been described as constituting a peripheral neuroendocrine system, analogous to the central neuroendocrine system of the hypothalamus[8].

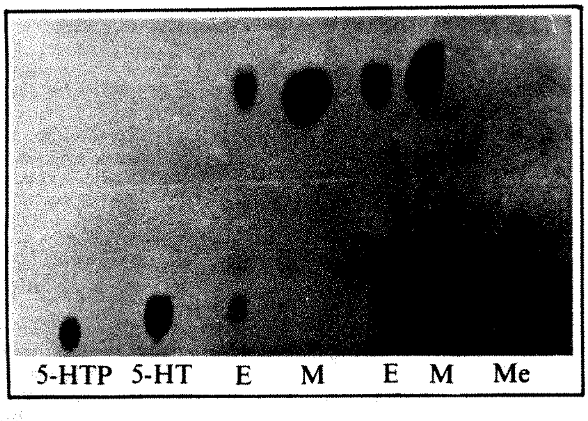

Fig. 2 Thin-layer chromatography of an alkaline extract of the intestinal mucosa of the rabbit. Chromatography was performed on Silufol UV 254 (Kavalier, Czechoslovakia) using isopropyl alcohol–benzyl alcohol–ammonia (10:5:1) as the solvent system. 5-HTP, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine; E, extract; M, melatonin; Me, 5-methoxytryptamine.

Table 1 Time changes in clarification effect depending on the number of enterochromaffin cells in mucosal lining of appendices used in preparation of alkaline extracts

State of appendix	No. of appendices	Average number of enterochromaffin cell per crypt*	Time of clarification
Acute cattarhal appendicitis	61	4–5	25–30 min
Acute phlegmonous appendicitis	95	16–30	15–20 min
Acute gangrenous appendicitis	17	0–2	No effect

*The number of enterochromaffin cells was counted in ten sections, taken from different regions of each appendix; in each section the number of enterochromaffin cells were counted in ten crypts—thus in each appendix we counted cells in 100 crypts. The number of cells thus counted was divided by 100 to obtain the number of enterochromaffin cells per crypt.

We thank Professors N. N. Suvorov and M. N. Preobrazhensky for chromatographic samples and for discussions.

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Received January 23; accepted March 14, 1975.

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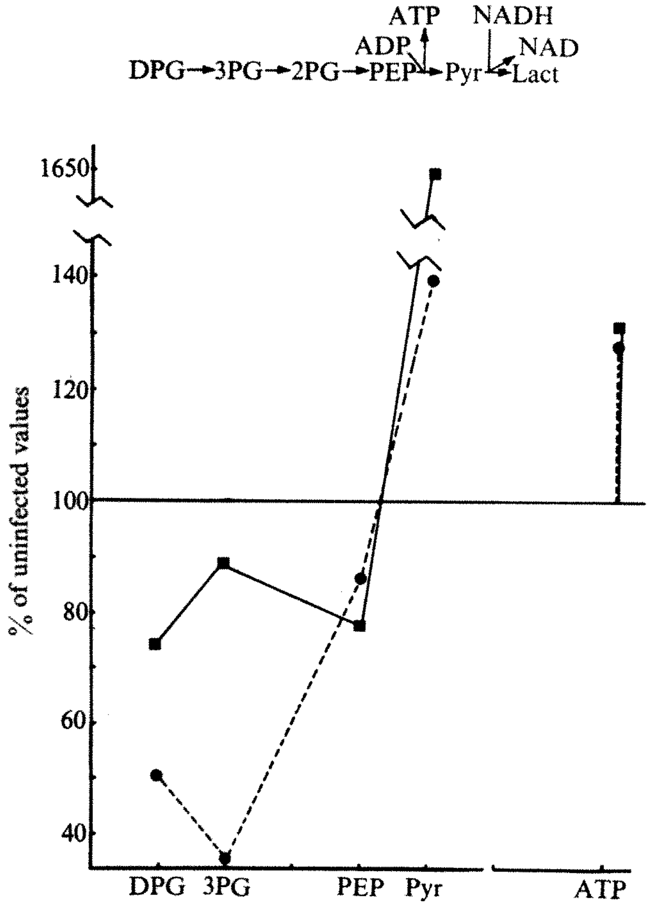
Pyruvate kinase in malaria host–parasite interaction

THE mammalian malaria host–parasite systems seem particularly useful for studies aimed at elucidating the biochemical mechanisms of such interactions. During the vertebrate phase of their life cycles the malaria organisms are intracellular parasites of the red cell. The mature mammalian host red cell is also relatively simple metabolically; its energy metabolism is solely that of Embden–Meyerhof glycolysis and it also lacks the capacity for protein synthesis. We therefore studied several aspects of red cell glycolysis in monkeys heavily infected with *Plasmodium knowlesi* and mice heavily infected with *P. berghei*. In both these systems we have found an increase in red blood cell adenosine triphosphate (ATP) and a decrease in red cell 2,3-diphosphoglycerate (DPG). We also have evidence that these malaria parasites introduce a pyruvate kinase isozyme into their host red cells in amounts sufficient to alter red cell glycolysis. First, glycolytic intermediate data demonstrate an *in vivo* increase in pyruvate kinase activity in infected red cells; second, there is an increase in pyruvate kinase V_{max} activity in infected cells; and third, gel electrophoretic patterns show a new pyruvate kinase isozyme in infected cells. We suggest that this alteration in red cell glycolysis is in a direction favourable



to the parasite because it will increase red cell ATP at the expense of red cell DPG.

Figure 1 summarises the glycolytic intermediate data in the form of glycolytic plots for the monkey–*P. knowlesi* and the mouse–*P. berghei* systems. The assay and extraction procedures have been described previously[1]. Glycolytic plots are subject

Fig. 1 Glycolytic plots of malaria-infected red cells. ———, Glycolytic plot obtained using values on four rhesus monkeys heavily infected with *P. knowlesi* (an average of 40% of red cells infected). The glycolytic intermediates are listed on the horizontal axis in their order of appearance in the glycolytic pathway. For each monkey the level of each intermediate is expressed as a ratio (%) of the monkey's preinfected level. The average percentage for all four monkeys is then plotted to give the solid line in the figure. Control monkeys, similarly bled and treated (including one bled to the same degree of anaemia as his counterpart)—but not infected—do not give this pattern. ------, Comparison of the intermediate levels obtained from the pooled blood of three *P. berghei* infected mice (64% of red cells infected) with the pooled blood obtained from three healthy mice. The small blood volumes obtainable did not enable us to assay the same mice both before and after infection as was done with the monkey. The extraction and assay procedures have been described previously[1].

to variable interpretations and in considering such plots it is useful to know whether glucose consumption has increased or decreased. We have found a 7.5-fold and a 12-fold increase in glucose consumption in *P. knowlesi*-infected monkey red cells (34% infected) and in *P. berghei*-infected mouse red cells (38% infected) respectively. (Similar glucose consumption data abounds in the malaria literature.) With these considerations the simplest interpretation of the glycolytic plots of Fig. 1 is that malaria infection leads to an increase in the *in vivo* activity of pyruvate kinase. Such an increase would lead to a decreased steady-state level of phospho-enol pyruvate (PEP) and preceding intermediates (Fig. 1). The increase in pyruvate and lactate seen in the plots would also be consistent with increased pyruvate kinase activity but since these two intermediates can pass through the red cell membrane (unlike PEP and preceding intermediates) and can arise from other sources (such as muscle), the levels of these two intermediates may not be as directly associated with red cell glycolysis. We and others have previously found that an increase in *in vivo* pyruvate kinase activity does lead to patterns similar to those seen in Fig. 1 including the decreased 2,3-DPG and increased ATP[2,3]. The mechanism by which these changes occur has been discussed previously[3].

Table 1 summarises the increases in pyruvate kinase activity (V_{max}) we have seen in malaria infected blood haemolysates. Clearly, enzyme activity is increased in infected blood. The

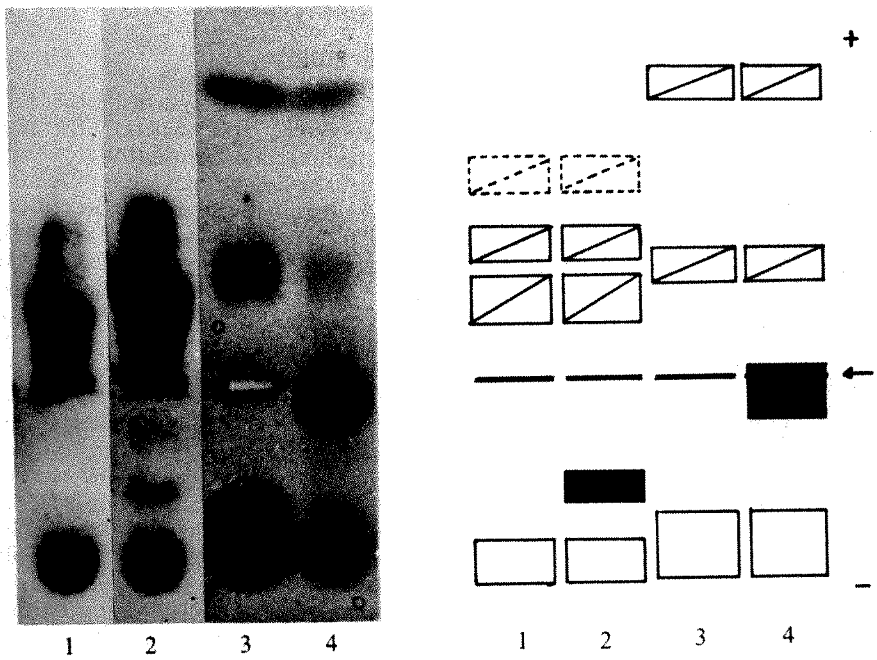


Fig. 2 Starch gel electrophoretic study of pyruvate kinase isozymes found in malaria-infected and uninfected blood samples. The adenylate kinase bands were discerned by staining the opposite face of the gel. This is necessary since the pyruvate kinase stain also stains for adenylate kinase[5]. In the diagram the dark bands are those which stained for pyruvate kinase activity only, the lined bands stained for adenylate kinase, whereas the open bands are haemoglobin bands. The dashed lines represent faint bands which are difficult to see in the photograph. Slot 1, uninfected monkey haemolysate; Slot 2, infected monkey haemolysate; Slot 3, uninfected mouse haemolysate; Slot 4, infected mouse haemolysate. In both the malaria infected mouse and monkey bloods a new pyruvate kinase isozyme can be seen. Presumably, as with human haemolysates, the uninfected mouse and uninfected monkey red cell pyruvate kinase activities are buried within the adenylate kinase area[5].

Table 1 Increase in pyruvate kinase V_{max} activity in malaria-infected red cells

Monkey–*P. knowlesi* system	% Parasitaemia*	% of Uninfected†
I	54	192
II	46	130
III	34	122
IV	26	119
Mouse–*P. berghei* system		
I	> 80	244
II	> 80	480
III	51	236

* % red cells containing parasite(s).

†Calculated as; 100 × (Infected V_{max})/(Uninfected V_{max}). Uninfected values are the same types as discussed in Fig. 1. The four control monkeys of the above experiments showed no or little increase (103 %) in pyruvate kinase activity.

pyruvate kinase assay was performed on lysates of red cells washed three times in saline with the buffy coat removed at each washing[4]. Activity was calculated on a per gram haemoglobin basis.

Figure 2 illustrates banding patterns obtained when the above infected and uninfected red cell haemolysates were subjected to starch gel electrophoresis and stained for pyruvate kinase[5]. It can be seen that in both *P. knowlesi* and *P. berghei*-infected blood cells a new band of pyruvate kinase activity is present which was not present in uninfected blood. Presumably these new enzyme bands are of parasitic origin. (Indeed, mature mammalian red cells lack the metabolic machinery to synthesise new enzymes.)

There has been much interest in the role of ATP in malaria infections. In human volunteers inoculated with *P. falciparum* the rate of parasitaemia was directly correlated with the individual's red cell ATP value[6]. Also, in monkeys infected with *P. cynomolgi* the maximum parasitaemia correlated with red cell ATP level[7]. In *in vitro* culture ATP favours the growth of the parasite[8]. Red cell ATP levels have been shown to vary during the course of infection with the magnitude and direction of change depending, perhaps, on the host–parasite system under study and stage of parasitic development during sampling[9–12]. Our studies have demonstrated an increase in ATP levels in heavily infected red blood cells for the mouse–*P. berghei* system and the monkey–*P. knowlesi* system. Pyruvate kinase is also implicated as a major determinant of this increase.

The source of ATP in the mature mammalian red cells is anaerobic glycolysis (such red cells cannot carry out oxidative phosphorylation). From a teleologic (and evolutionary) viewpoint the introduction of pyruvate kinase into the red cell by the parasite is biochemically sound. Pyruvate kinase is a rate-limiting step of red cell glycolysis and a relative increase in pyruvate kinase activity is known to increase red cell ATP at the expense of DPG[2,3]. ATP is needed and/or used by the parasite, while DPG is important for red cell oxygen transport function but presumably is of no value to the parasite (ATP and DPG are the two major intermediates in monkey and mouse red cells.)

Although our study demonstrates a role for pyruvate kinase in at least two mammalian malaria host–parasite systems, it certainly does not exclude other concurrent enzyme interactions. Certainly, the large increases in glucose consumption cannot be explained simply on the basis of a pyruvate kinase increase. Other factors most certainly must be operative. It would be interesting, however, to see if similar pyruvate kinase data exist for other host–parasite systems. One species of avian malaria also seems to have its own pyruvate kinase isozyme[10]. Perhaps *in vivo* pyruvate kinase perturbations are a general host–parasite phenomenon.

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Received January 30; accepted March 18, 1975.

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NMR evidence for common tertiary structure base pairs in yeast and *E. coli* tRNA

ABOUT ten years ago Holley[1] and coworkers sequenced the first tRNA and proposed the now famous cloverleaf secondary structure. Since that time about sixty additional tRNAs have been sequenced and all fit the cloverleaf model[2]. This homology constitutes a strong case for the cloverleaf model and there is now compelling evidence from nuclear magnetic resonance (NMR) studies[3] that the cloverleaf model does provide a correct description of the secondary structure of tRNA molecules in solution.

In addition to secondary structure homologies, tRNAs also contain constant bases in single stranded regions of the cloverleaf model. The first of these homologies was pointed out by Levitt who noted that residues in positions 15 and 48 are either G_{15}–C_{48} or A_{15}–U_{48} (ref. 4). On this basis Levitt suggested that bases in positions 15 and 48 form a tertiary structure base pair. The accumulation of tRNA sequences since Levitt first made his proposal has shown that other homologies exist in Class I (ref. 4) (D4V5) (ref. 5) tRNA, including A_{15}–U_8 (S^4U_8), G_{19}–C_{56} and A_{58}–T_{54} (refs 4–6) and hence these too may be involved in tertiary structure base pairs.

In the light of these observations we are interested in the possibility of detecting the presence of additional base pairs beyond those predicted by the cloverleaf model and have recently shown that an anomalous 14.8 p.p.m. resonance which occurs in the low field NMR spectra of many *Escherichia coli* tRNAs is a result of a common homologous tertiary structure base between A_{14} and S^4U_8 (refs 7 and 8). The fact that we were able to identify unequivocally a resonance from one tertiary structure base pair suggested that it might be possible to observe resonances from other tertiary structure base pairs.

To test the proposal that most Class I tRNAs contain an identical set of tertiary structure base pairs, we have examined the low field NMR spectra of unfractionated *E. coli* and yeast tRNA at several different temperatures with and without magnesium. The idea behind these experiments was quite simple. Since the proposed tertiary base pairs are the same or nearly the same in all Class I tRNA they should give rise to relatively sharp resonances and thus should stand out above the broad background of unresolved overlapping resonances from secondary structure base pairs. Futhermore, resonances from tertiary structure base pairs should be more temperature sensitive than secondary structure base pairs. The NMR spectra of unfractionated *E. coli* and yeast tRNA are shown in Figs 1 and 2 and they do exhibit several sharp resonances in the low field region. To determine whether or not these resonances correspond to tertiary structure base pairs, the effect of salt and tem-

Table 1 Comparison of the homologous bases in *E. coli* and yeast tRNA

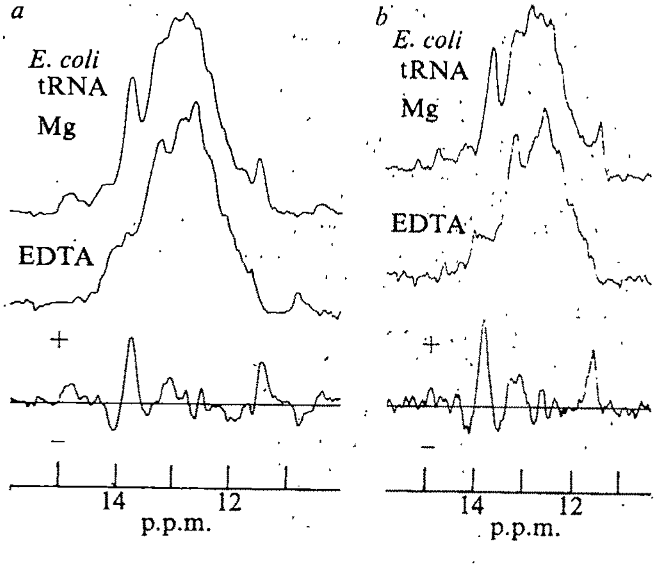
	E. coli Class I (D4V5)	All	Yeast Class I (D4V5)	All
8–14	S^4U–A (12/13) U–A (1/13)	S^4U–A (16/21) U–A (5/21)	U–A (7/8) U–G (1/8)	U–A (13/14) U–G (1/14)
19–56	G–C (13/13)	G–C (21/21)	G–C (8/8)	G–C (14/14)
15–48	G–C (11/13) A–U (2/13)	G–C (15/21) A–U (5/21)	G–C (2/8) A–U (3/8) G–m^5C (2/8) U–C (1/8)	G–C (3/11) A–U (4/11) G–m^5C (3/11) U–C (1/11)
54–58	A–T (13/13)	A–T (21/21)	A–T (2/8) m^1A–T (6/8)	A–T (6/14) m^1A–T (8/14)
9–12–23*	A–U–A (12/13) G–C–G (1/13)	———	A–U–A (2/8) m^1G–C–G (4/8) m^1G–U–A (2/8)	———
13–22–46*	C–G–M^7G (12/13) C–G–A (1/13)	———	C–G–m^7G (4/8) C–G–G (2/8) Ψ–A–m^1G (1/8) U–A–A (1/8)	———

The numbering system is that of yeast $tRNA^{Phe}$. Sequence data were obtained from ref. 2. Sequences included in Class I (D4V5) are for *E. coli*; Arg Asp$_1$, Gly$_3$, His$_1$, Ile$_1$ Met$_f$ Met$_m$ Phe, Trp, Val$_1$, Val$_{2a}$ and Val$_{2b}$ and for yeast; Ala, Arg$_3$, Gly$_1$, Lys$_1$, Lys$_2$, Met$_f$, Phe and Trp.
* Those tRNAs which are not Class I do not exhibit homology in the base triples 9–12–23 and 13–22–46.

perature on the spectra were investigated.

The spectra presented in Fig. 1 clearly show that magnesium has a pronounced effect on the low field spectrum of unfractionated *E. coli* tRNA. The difference in the spectrum with and without magnesium at 50 °C shows pronounced peaks at 13.8, 13.0 and 11.8 p.p.m. The most striking feature of the difference spectrum in Fig. 1 is its sharpness. This indicates that there is a magnesium effect on the tRNA structure and that the change is the same for most or all of the tRNAs. In light of the variability in the secondary structure base pairs it would seem that a common change in secondary structure of all tRNA cannot be responsible for this effect. Integration of the peaks in the difference spectrum shows that the amount of change increases from 24 to 50 °C, such that at 50 °C the intensity of each of the three main peaks in the difference spectrum corresponds to about 0.9 protons (assuming the intensity of the low field spectrum in the presence of magnesium corresponds to 22 protons per molecule) (determined by comparison of the integrated intensity of the aromatic and low-field resonances in H_2O and D_2O). The two main conclusions to be drawn from these data are that the spectra changes arise from base pairs which are common to most or all tRNA and that these are the weakest base pairs in the structure.

Fig. 1 All spectra were measured at 300 MHz with a Varian HR300 spectrometer operated in the field sweep mode. The signal from the spectrometer was averaged by a Nicolet 1020A computer. The temperature was controlled to ±1 °C during the course of an experiment. Positions are in p.p.m. downfield from 2,2-dimethyl-silapentane-5-sulphonate (DSS). *E. coli* unfractionated tRNA was obtained from Plenum Scientific (Lot 74121). The samples were prepared by dialysis against 0.1 M NaCl, 10 mM cacodylate *p*H 7.0 and 2 mM EDTA. The tRNA concentration was 35 mg ml^{-1} in a sample volume of 10 µl. *a*, NMR spectra of *E. coli* unfractionated tRNA at 40 °C with Mg (Mg) and without (EDTA). The difference spectrum is that of the sample containing magnesium minus the one containing EDTA. *b*, NMR spectra of *E. coli* unfractionated tRNA at 50 °C with Mg (Mg) and without (EDTA). The difference spectrum is calculated as in *a*.



The behaviour of unfractionated yeast tRNA is basically the same as *E. coli* tRNA (see Fig. 2) although the peaks in the difference spectra are not as sharp as in *E. coli*. This is probably a result of the greater variance in the homologous base pairs as indicated in Table 1. To check the overall intensity change induced by the magnesium we measured the spectrum of a sample with magnesium and then again after the EDTA had been added to remove the magnesium. The sample was strongly buffered to eliminate *p*H changes resulting from the EDTA addition and all spectra were taken with identical instrument settings. Comparison of the two spectra showed a slight loss of intensity 2±1 produced by the removal of the magnesium at 40 °C. The uncertainty in the net loss introduces a slight uncertainty as to where the base line of the difference spectrum is to be drawn, but does not affect the appearance of the sharp differences which are observed.

The peaks in the difference spectrum share two important characteristics which indicate that they should be attributed to tertiary structure base pairs: first, common resonance positions in the low field spectrum indicating they correspond to base pairs which are common to most of the tRNA and second, early melting indicating they are the weakest base pairs in the molecule. On the basis of position and intensity we tentatively assign the peak in the difference spectrum at 13.8 p.p.m. to the invariant A–T base pair in the TψC loop and the peak at 13.0 p.p.m. is assigned to the invariant G_{19}–C_{56} base pair. These assignments are consistent with the ring current shift parameters[8] which we have successfully used to interpret the low field resonances arising from secondary structure base pairs and with other factors discussed below. The peak at 11.8 is assigned to a common G_{22}–C_{13} secondary structure base pair and broadening of this resonance results from the loss of tertiary structure and unstacking of A_{14} from G_{22}–C_{13}.

We also considered assigning the difference spectrum to

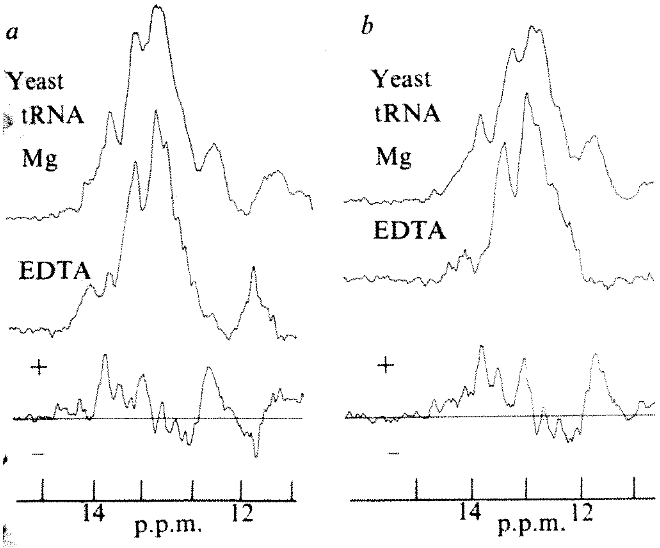


Fig. 2 The spectra were obtained and the sample prepared as described in Fig. 1. Yeast unfractionated tRNA was obtained from Plenum Scientific (Lot 72041). *a*, NMR spectra of yeast unfractionated tRNA at 40 °C with Mg (Mg) and without (EDTA). The difference spectrum is that of the sample containing magnesium minus that of the one containing EDTA. *b*, NMR spectrum of yeast unfractionated tRNA at 50 °C with Mg (Mg) and without (EDTA). The difference spectrum is calculated as in *a*.

loss of the DHU stem since it too is highly homologous in Class I tRNA and is expected to be the weakest secondary structure helix in the molecule. Such an assignment seems unlikely, however, for the following reasons. First, the intensity distribution of the difference spectrum (with the exception of G_{22}–C_{13}) does not fit that predicted by ring current calculations on the DHU stem. Second, we have observed that tRNA fragments containing the DHU stem do not melt in the absence of magnesium until above 50 °C (B. R. Rordoff and D. R. Kearns, in preparation). Third, only about 50–60% of the *E. coli* tRNA have appropriate DHU stem sequences whereas the intensity loss at 13.8 corresponds to one resonance per molecule. Finally, the NMR spectra of various other pure tRNAs (*E. coli* $tRNA^{Arg, Val_1, Phe, Asp_1}$ and yeast $tRNA^{Phe}$ and others) exhibit anomalously high intensity at 13.8 p.p.m. the position where the intensity in the difference spectrum in the unfractionated tRNA is greatest. On the basis of these and other observations to be described elsewhere, we conclude that two of the main features observed in the *E. coli* and in the yeast difference spectra (magnesium minus no magnesium) correspond to tertiary structure base pairs. These results, taken with our earlier studies of the base pairing involving S^4U_8 indicate that there are at least three tertiary structure base pairs present in tRNA in solution. If our proposed assignments are correct, these would correspond to three of the same tertiary base pairs which have been proposed on the basis of X-ray diffraction studies of yeast $tRNA^{Phe}$ (refs 10 and 11). It would also seem that the tertiary structure base pairing and hence the tertiary structure of most *E. coli* as well as yeast tRNA are nearly identical. This provides strong justification for generalising features observed for one Class I (D4V5) tRNA to others.

The support of the US Public Health Service is acknowledged.

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Received February 25; accepted March 26, 1975.

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Ultraviolet inducible protein associated with error prone repair in *E. coli* B

Escherichia coli is affected in many ways by irradiation with ultraviolet light. New DNA is made containing gaps resulting from synthesis on damaged templates[1,2] and postreplication repair seals them by exchanging sections of DNA between daughter duplexes[3,4]. The induction of mutations by ultraviolet light has been attributed to errors occurring during this type of repair[5–7] and so this repair has been called error prone. Ultraviolet radiation also results in prophage induction[8], inhibition of respiration[9], filamentous growth[10] and the development of extra capacity to promote the survival[11] and mutagenesis[12] of irradiated infecting phages. All these events require the *recA*$^+$ and *exr*$^+$ genes[2,7,9,13–23] but the ways in which *recA*$^+$ and *exr*$^+$ gene products affect such a diversity of postirradiation events are not known. One proposal is that they act in a single molecular process which in some way initiates all the phenomena described above and that the *recA*$^+$ *exr*$^+$ dependent functions are inducible[23] by ultraviolet light and thus require the synthesis of new protein(s) in irradiated bacteria. Here I demonstrate that *E. coli* does indeed synthesise a protein in response to ultraviolet irradiation and that the synthesis of this protein is dependent on *exr*$^+$ and *recA*$^+$ genes.

Cultures of *E. coli* strains WP2$_s$ *uvrA trp*[24], and its conjugal or transductional derivatives WP100 *uvrA recA trp* and CM611 *uvrA exrA trp*[25] were grown overnight, diluted 100-fold into Bacto Davis minimal medium supplemented with tryptophan (20 μg ml^{-1}) and grown to cell densities of about 10^8 per ml. The proteins synthesised in unirradiated and ultraviolet-irradiated bacteria were labelled for 20 min by the addition of 2 μCi of ^{35}S-methionine (specific activity 149 Ci mmol^{-1} obtained from New England Nuclear Corp.) and 0.02 μg of methionine to 0.1 ml aliquots of culture. The cells were pelleted, lysed in 25 μl of 0.05 M Tris-HCl (*p*H 6.8), 1% sodium dodecyl sulphate, 1% mercaptoethanol, 0.002 M EDTA, 10% glycerol and 0.025% Coomassie blue and heated in a boiling water bath for 2 min. This treatment causes protein denaturation and dissociation, giving a preparation of unassociated individual proteins and protein subunits of multimeric molecules.

The proteins were analysed by electrophoresis through 10% polyacrylamide gels containing 0.26% N,N′-methylene *bis* acrylamide, 0.19 M Tris-HCl (*p*H 8.8), 0.1% sodium dodecyl sulphate, 0.002 M EDTA. The electrode buffer contained 0.05 M Tris, 0.38 M glycine, 0.1% sodium dodecyl sulphate and 0.002 M EDTA. Electrophoresis was for 75 min at 200 V using the apparatus and procedure described by Studier[26]. Autoradiograms of dehydrated gels were made using Kodak SB54 X-ray film. A typical autoradiogram is shown in Fig. 1.

Columns *a*, *c* and *e* show the identical distributions of proteins from unirradiated strains WP2$_s$ *uvrA*, WP100 *uvrA recA* and CM611 *uvrA exrA*, respectively. An ultraviolet dose of 4.8 J m^{-2} to WP2$_s$ specifically induced the synthesis of the protein indicated by the arrow in column *b*. There was no induction of this protein in irradiated bacteria lacking either *recA*$^+$ (column *d*) or *exr*$^+$ (column *f*) genes. The overall pattern of other proteins in all the strains was not influenced by ultraviolet radiation.

The phenomena induced by ultraviolet radiation were also induced after incubating bacteria with the *tif* mutation at 42 °C[7,27–29]. The proteins of the strain WP44$_s$-NF *uvrA*

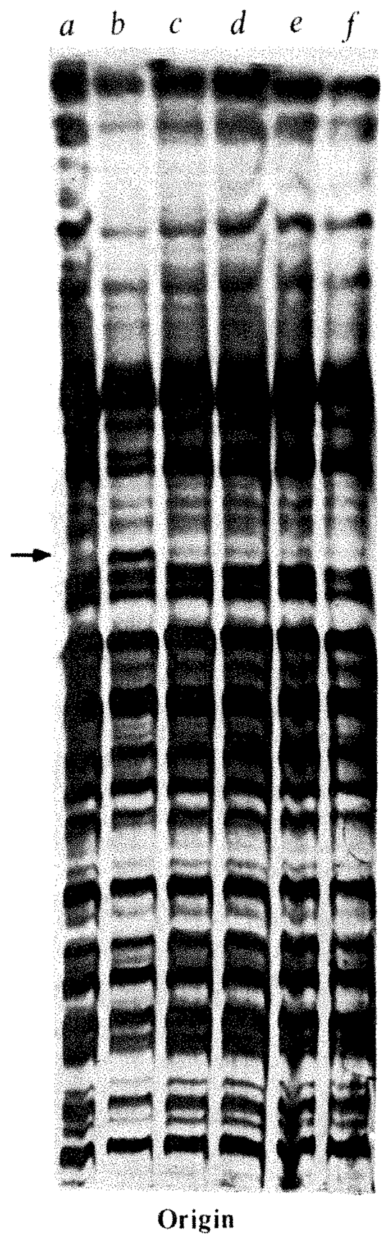

Fig. 1 Proteins made in unirradiated and ultraviolet-irradiated *E. coli*. Proteins were radioactively labelled with ^{35}S-methionine and analysed by electrophoresis through 10% polyacrylamide as described in the text. The proteins shown are from strains: *a*, WP2$_s$ *uvrA*, no ultraviolet; *b*, WP2$_s$ *uvrA*, 4.8 J m^{-2} ultraviolet; *c*, WP100 *uvrA recA*, no ultraviolet; *d*, WP100 *uvrA recA*, 4.8 J m^{-2} ultraviolet; *e*, CM611 *uvrA exrA*, no ultraviolet; and *f* CM611 *uvrA exrA*, 4.8 J m^{-2} ultraviolet. The arrow indicates the position of a protein band only present in irradiated *E. coli* WP2$_s$ *uvrA*.

tif were analysed after growth at inducing, 42 °C, or non-inducing 30 °C temperatures. Incubation at 42 °C increased synthesis of the same new protein seen in irradiated *E. coli* WP2$_s$ *uvrA*.

I conclude that events such as error prone repair and filamentation are inducible because they are caused by the activity of *recA*$^+$ and *exr*$^+$ genes, which are shown here to allow the induced synthesis of a specific new protein in irradiated cells.

I thank Drs F. W. Studier and G. McGovern for help and Dr G. M. Witkin for WP100 *uvrA recA trp* and WP44$_s$-NF *uvrA tif*. The research was carried out under the auspices of the US Energy Research and Development Administration.

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Histones in fixed cytological preparations of Chinese hamster chromosomes demonstrated by immunofluorescence

HISTONES and DNA make up the bulk of the chromosomal material and are presumably present in approximately a 1 : 1 ratio. Although it is known that histone fractions elicit specific antibodies[1]; a crude basic nuclear protein will bind to chromosomes[2]; antisera against lysine-rich histones will stain nuclei[3]; and purified histone antisera will bind to chromatin[4], little is known about the distribution of histones in fixed preparations of chromosomes. We used the immunofluorescent technique to show that an antiserum prepared from the F3 histone fraction of human leukaemic lymphoblasts binds to both human and *Drosophila* salivary gland chromosomes[5]. We have now made antisera against the five major histone fractions of calf thymus and characterised them by microcomplement fixation[6]. Using selected antisera, we found that all except the F1 histone antiserum bind to acid-fixed Chinese hamster chromosomes to various degrees. Furthermore, in some preparations a pattern of transverse banding appears. We have also found that histones remaining on the chromosome after acid fixation are very firmly bound, which is necessary for the credibility of our observations.

Calf thymus histones were fractionated by the Johns[7,8] techniques and examined by amino acid analysis and polyacrylamide gel electrophoresis[9]. Antisera were induced by dissolving 5 mg lyophilised histone fraction in water, emulsifying in an equal quantity of Freund's complete adjuvant and injecting into multiple sites on the backs of mature female New Zealand rabbits once per week for 6 weeks (Fig. 1). Many rabbits had a repeat immunisation after 2 months to raise complement fixation titre further.

Chinese hamster chromosomes (Don and Dede lines, American Type Culture Collection) were prepared by standard acid fixation using methanol–acetic acid (3 : 1). Histone antiserum binding was assessed by indirect immunofluorescence (Fig. 2). Some acid-fixed slides were treated with 0.2 N HCl for 15 min, 1, 2 and 4 h before exposure to antisera and immunofluorescence (Fig. 3).

Immunisation of rabbits with the F1, F2a1, F2a2, F2b and F3 histone fractions provided antisera with titres of 1 : 250 to 1 : 1,000 by microcomplement fixation. Antisera from all except the F3 fraction could be obtained from

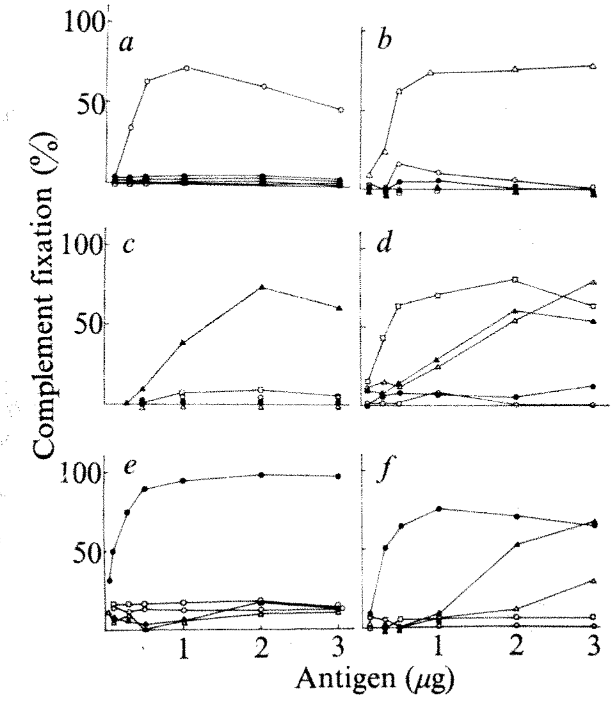

Fig. 1 Microcomplement fixation reaction of histone fractions F1 (○), F2a2 (△), F2b (▲), F3 (□) and F2a1 (●) with rabbit antisera induced by these fractions. *a*, F1 histone antiserum diluted 1:1,000; *b*, F2a2 histone antiserum diluted 1:250; *c*, F2b histone antiserum diluted 1:500; *d*, F3 histone antiserum diluted 1:1,000; *e*, F2a1 histone antiserum, 7S fraction diluted 1:50 (original titre of antiserum 1:500) and *f*, F2a1 histone antiserum diluted 1:250.

some rabbits that did not cross react with the other histone fractions (Fig. 1). In other rabbits significant cross reactivity was noted on either primary or repeated immunisation.

Antisera derived from all except the F1 histone fraction bound to the Chinese hamster chromosome. The F1 histone antiserum, although of high titre and specificity, did not bind appreciably even to bovine chromosomes. Antisera induced by the F2a1 histone fraction have been studied most extensively to date. That of Fig. 1*e* had an original titre of 1 : 500 and, as shown in the figure, did not cross react even when the 7S fraction of this antiserum was concentrated 1 : 50. The F2a1 antiserum shown in Fig. 1*f* cross reacted mainly with F2b at higher concentrations of antigen. Because the latter antiserum gave a crisper segmental pattern most preparations shown here were exposed to it. Evidence that these antisera were directed against histones comes from the fact that one or the other of them showed (1) absence of DNA antibodies by radioimmunoassay, (2) lack of species specificity, (3) absence of staining of nucleoli in interphase cells and (4) lack of staining if *Drosophila* salivary glands were incubated with 0.2 N HCl to remove histones before fixation of chromosomes (L. P., unpublished).

Distinct transverse fluorescent bands appeared on some preparations treated with F2a1-rich histone antiserum (Fig. 2). Whereas contracted chromosomes stained homogeneously and brightly, those with elongated chromatids often showed bright banded areas which increased in number with elongation. The centromere did not stain. As with traditional banding techniques, preparations were influenced by the quality and age of chromosome spreads. There was also variation in the degree and pattern of staining on a given slide. Figure 4*a*–*d* shows four chromosomes No. 1 from different metaphase preparations well stained with F2a1-rich histone antiserum. Figure 4*a*–*c* shows variations in binding but also some agreement in binding patterns in areas where chromosomes were similarly elongated. Figure 4*d* shows a somewhat spiral arrangement. The grouping of similarly arranged chromosomes in Fig. 4*e* and *f* is an attempt to determine whether the pattern of a chromosome well stained with non-cross-reactive F2a1 antiserum (Fig. 4*e*) resembles that of a G-banded chromosome (Fig. 4*f*). Many more such pairs must be examined before a definitive judgment can be made on resemblance to any existing banding pattern.

At a given dilution, non-cross-reactive F2a2 and F2b histone antisera did not stain as well as F2a1 antisera. Banding was fainter with the former whereas the latter created a more general, softer pattern. The highly cross-reactive F3 histone antiserum gave a brilliant banding pattern. Whereas F2a1 and F3 antisera could be used satisfactorily at a 1 : 48–1 : 72 dilution, F2a2 and F2b antisera stained best at a 1 : 12 dilution. F1 antisera demonstrated little or no fluorescence even at 1 : 6.

Incubation with 0.2 N HCl before immunofluorescence with the F2a1-rich histone antiserum decreased the intensity of overall fluorescence slightly. After 15 min, detail was blurred and by 4 h a somewhat sharper banding pattern emerged (Fig. 3).

There is much speculation about the effects of acid fixation on the presence of histones on chromosomes. Estimates of removal range from 7 up to 8% of histones from chromatin preparations (predominantly F2a1 and some F2a2 and F3)[10] to 80% of histones from interphase cells (leaving F1 and F2b)[11] to some of all histones from nuclei[12]. But these previous studies involved chromatin or interphase nuclei, rather than metaphase chromosomes to which histones bind much more firmly[13].

Our finding that antisera against four of the five major histone fractions all bound to some degree to the chromosome suggest that there is a significant amount and variety of histones left on the chromosome after acid fixation. The F2a1-rich histone antiserum, studied in the greatest detail so far, gave especially brilliant transverse bands on some metaphase preparations. The non-cross-reactive F2a1 anti-

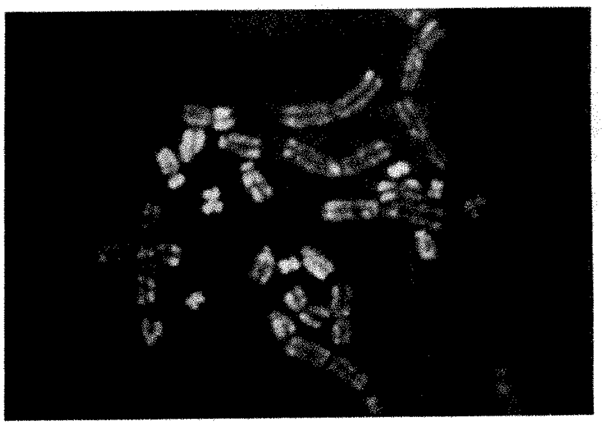

Fig. 2 Chinese hamster chromosomes prepared by standard acid fixation and stained by indirect immunofluorescence using F2a1-rich antiserum (1:24) and rhodamine-conjugated IgG fraction of goat anti-rabbit gamma globulin 1:30 (Cappel Laboratories). Chromosomes were fixed with methanol–acetic acid (3:1) and air dried on slides after exposure for a brief period on a warming plate. They were stored for short periods at 4 °C. The immunofluorescent reaction was as follows. Slides were flooded with antiserum against the histone fraction to be studied, incubated in a humid chamber at 37 °C for 30 min and rinsed with phosphate-buffered saline (PBS). After air drying, the slides were flooded with either rhodamine-conjugated IgG fraction of goat anti-rabbit gamma globulin (Cappel Laboratories) or fluorescein-conjugated antibody globulin to rabbit 7S gamma globulin (Hyland Laboratories). Both conjugates were satisfactory at a 1:30 dilution. Incubation was as above, followed by PBS rinsing and air drying. Slides were then viewed in the Leitz Ortholux microscope equipped with the fluorescence vertical illuminator with appropriate filters for fluorescein or rhodamine.

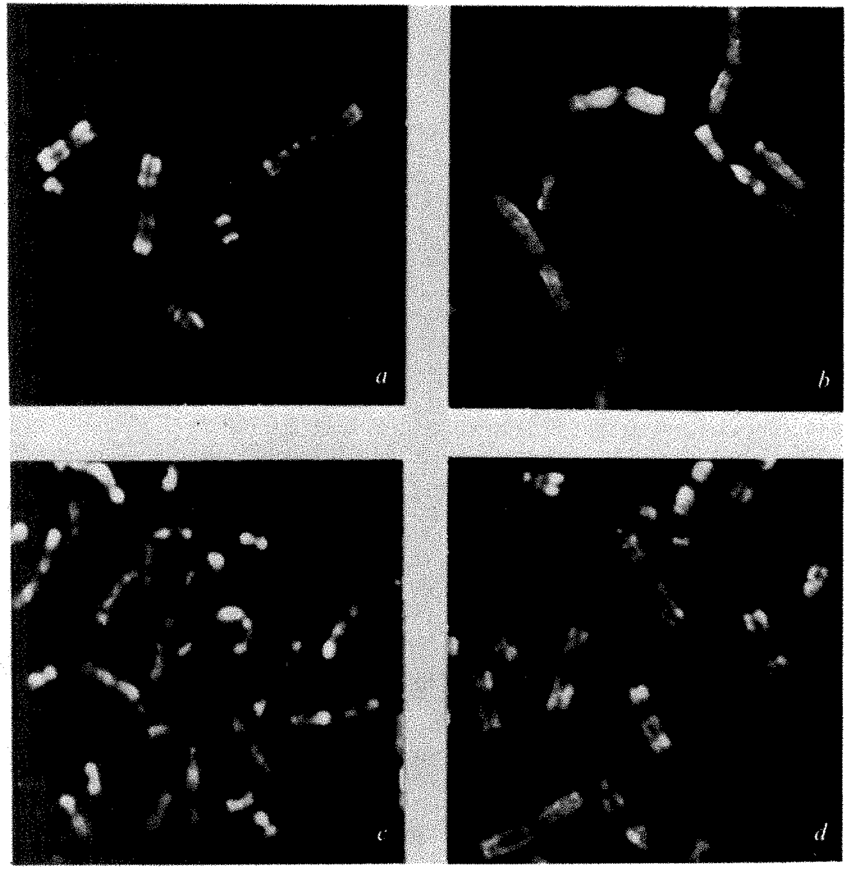

Fig. 3 The effect on immunofluorescent reaction with F2al-rich histone antiserum of prior treatment of chromosomes with 0.2 N HCl for: *a*, 0 h (control); *b*, 15 min; *c*, 1 h; and *d*, 4 h. (Histone antiserum diluted 1:24 and fluorescein-conjugated antibody globulin to rabbit 7S gamma globulin diluted 1:20.) All photographs were taken with an exposure of 15 s and from one experiment using the same batch of chromosomes.

serum stained similarly though less intensely. The weaker staining characteristics of F2a2 and F2b antisera may be more a reflection of the antigenicity of the histone fraction than the quantity of histone on the chromosome. The strong staining of F3 antiserum may be in part a manifestation of its cross-reactivity with the other histone fractions. The finding that bovine chromosome preparations could not improve the binding of the F1 histone antiserum suggests that this lack of binding was not caused by the inherent species specificity of this histone fraction, but rather to removal of F1 histone by acid fixation. This is likely since this fraction is easily removed by certain extraction procedures[14]. Of course, availability of binding sites of the various histone species on the chromosome as determined by steric factors, conformational changes or binding to other proteins will affect binding of all the antisera.

Acid-fixed chromosomes incubated with 0.2 N HCl for 4 h can still be Giemsa banded[12], and so it has been concluded that histones are not involved in banding, for the 0.2 N HCl extraction procedure reputedly removes all histones. Our finding that, after prior acid fixation of chromosomes, treatment with 0.2 N HCl for 4 h will not markedly reduce the immunofluorescent reaction with an antiserum against the F2a1 histone, suggests, however, that histones are not completely removed by this procedure. Whether histone binding will indeed relate to any specific banding pattern is unknown at present. But since a certain amount of histone is firmly bound to the chromosome by acid fixation, histones may modify banding patterns. It is more important tha observation of the binding sites of histone antisera directly on the intact chromosome should yield useful information about the spatial relationship of histone to DNA and the role of the various histone fractions in chromosome structure and function.

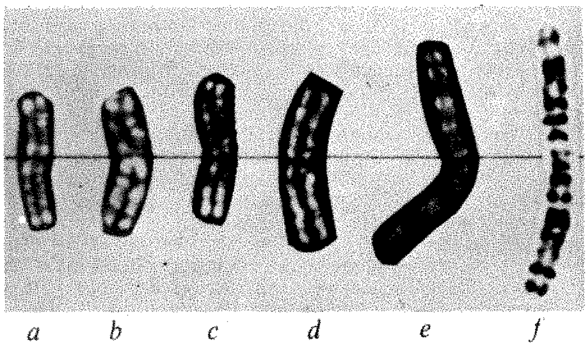

Fig. 4 Chinese hamster chromosome No. 1 selected from various acid-fixed metaphase preparations. *a*, *b*, *c*, *d*, *e*, Stained with antisera made against the F2al histone fraction using the immunofluorescent technique and (*f*) stained with the Giemsa banding technique (see text).

This work was supported by a grant from the National Cancer Institute. We thank Dr Martha Mattioli for the DNA antibody assays done in the laboratory of Dr Morr Reichlin, Departments of Medicine and Biochemistry, State University of New York, Buffalo.

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Received February 10; revised March 17, 1975.

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reviews

Extended view of evolution

A. Hallam

Evolution and Extinction Rate Controls. (Developments in Palaeontology and Stratigraphy, vol. 1.) By Arthur J Boucot. Pp. xv+425. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl. 110; $42.50.

For many years Professor Boucot has been both an indefatigable collector of Silurian and Devonian brachiopods over the whole world, and an equally energetic taxonomist. The vast amount of information he has compiled is summarised in this book which also includes a valuable survey of the important work on Palaeozoic marine invertebrate communities undertaken during the last decade by palaeontologists on both sides of the Atlantic.

It is anything, however, but a straightforward descriptive review. Right from the preface and introductory chapter Boucot plunges into his principal theme, which is basically simple: as is well exemplified by his brachiopods (though that principle is clearly thought to be of more general validity) evolution was most rapid when potentially interbreeding populations were small in size. Although other controls on rates of evolution are conceded they are dismissed as of relatively minor importance. In the course of his argument, by marshalling a wide array of facts, Boucot launches penetrating criticisms against hypotheses conflicting with his own, such as the Bretsky–Lorenz hypothesis which postulates an inverse relationship between rate of evolution and broad environmental tolerance (and genetic variability); and weaknesses in the widely accepted stability–time hypothesis of Sanders are also pointed out.

What then are we to make of Boucot's own hypothesis? Although it seems reasonable biologically, one is faced directly with the shortcoming of the method used to assess the population size of a given taxon, which is to estimate the area of distribution. It is not obvious to me that there should be such a simple correlation. Again, Boucot argues that cosmopolitan taxa evolved more slowly than provincial taxa because they had larger populations. Why could not that reflect simply the greater tolerance of the cosmopolitans, which were able to survive the minor environmental vicissitudes that evidently proved too rigorous for the provincials? When Boucot generalises he is forced to indulge in special pleading. Consider, for instance, the following highly dubious and unsupported statement on page 114: "The Mesozoic ammonoids . . . exploded into a tremendous development of both taxa and individuals during several post-Palaeozoic intervals that are consistent with the concept of population size being inversely related to rate of evolution" Mammals evidently evolved faster than invertebrates because their populations were smaller. Is there any evidence whatsoever that large mammals, with smaller populations, evolved faster than small ones? In my opinion the fossil evidence can more plausibly be held to support quite another view: that particular groups of animals, whether mammals, ammonites, bivalves or brachiopods, had characteristic rates of evolution regardless of the size of individuals, which usually correlates inversely with population size. Stanley's view that mammals evolved faster than bivalves because they competed more intensively seems much more reasonable.

In spite of his extensive discussion of biogeography Boucot holds a curiously agnostic position on plate tectonics, which is evidently regarded as being too speculative to warrant more than the most passing mention. I agree with his view that plate tectonics fails to explain many features of Silurian–Devonian provinciality. Why should the early Devonian faunas exhibit more provincialism, for instance, than those of the late Silurian, although the continental configurations had not changed significantly in the interim? It seems reprehensible, on the other hand, that Boucot neglects to consider a Gondwanaland configuration in his discussion of the Malvinokaffric Realm, which he bases on distinctive faunas occurring only in New Zealand, South Africa and southern South America.

A final comment on this interesting and provocative work concerns the rambling and verbose style of prose. If he had organised his material better and avoided the numerous repetitions he could have condensed his book into about half the length, thereby bringing the cost down to a level within the reach of individuals as opposed to institutions □

Intelligence book

Bernard Meltzer

Artificial Intelligence. (Academic Press Series in Cognition and Perception) By Earl B. Hunt. Pp. xii+468. (Academic: New York and London, January 1975.) $29.00; £13.90.

This introduction to artificial intelligence deals with a wider range of topics than is customary in such texts—computability, pattern recognition, heuristic, problem solving, automatic theorem proving, computer perception and comprehension of natural language. A newcomer who wants to know, for example, what an augmented transition network for the analysis of language is, or what inference by resolution means, would get some idea from this book.

But, alas, much of the exposition is neither clear nor correct. Explanations using *non-sequiturs* are particularly depressing, and inadequate diagrams obscure rather than illuminate them. The account of the work of Guzman, Clowes, Huffman on the perception of line diagrams of polyhedral solids, and the treatment of picture interpretation generally, is particularly poor—perhaps not so surprisingly: 24 pages are devoted to computer perception, whereas 161 consider 'classical' pattern recognition, the relevance of which to artificial intelligence is much smaller.

The treatment of theorem proving is marked by a confusion between the propositional and quantificational calculus, and little account is given of the important work on mathematical reasoning outside the resolution framework. The notions of analogy and model are confused with each other. Besides such obfuscations, the author is given to producing, out of the blue, large general judgments with little justification. □

Watch words for dilettanti

My Father's Watch. By Donald F. Holcomb and P. Morrison. Pp. xxvi+390 (Prentice-Hall: Englewood Cliffs, 1974) $11.50.

THIS enjoyable book was written for students having no professional interest in science. The authors have aimed at producing a book which is essentially 'core material' for a one-year course in physics for this type of student, supplemented by a sketch of a number of special topics. If one were to base a course on the book, quite a bit of flexibility could be attained by varying the emphasis placed on the topics covered —a nice feature.

Part 1 of the book—"The way things are"—starts with "Looking at the sky", goes on to discuss the measurement of length and time, geometry and the real world and concludes with a consideration of "exponential processes in nature"; in that chapter, a mathematical model for population growth is discussed alongside radioactive dating and the whole approach is novel and stimulating. Part 2 is concerned with "motion", and Part 3 with "energy". About 115 pages are devoted to the latter topic out of a book of some 400 pages and among the special topics "energy and man" also figures, with a good general bibliography.

The authors are well known experts in presenting physics and the illustrations of the material in the book are highly original and often very beautiful. Thus, to illustrate translation symmetry we are shown the facade of the Doge's palace in Venice along with a row of Persian archers sketched from a frieze in Darius' Palace in Susa.

Finally, the title of the book—it is taken from the poem by John Ciardi. In the prologue the authors write "The Watchmaker turns out to be far more subtle than any who practice that skilled and yet rather limited trade; He is not to be found in the Watch (≡ the Universe), or even peering through the glass".

The authors have written an exceptionally fine book of its kind, which deserves success.

N. H. March

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Amino acid disorder

Heritable Disorders of Amino Acid Metabolism: Patterns of Clinical Expression and Genetic Variation. Edited by William L. Nyhan. Pp. xvii+765. (Wiley: New York and London, December 1974.) £17.25.

IT is traditional in any book on inborn errors of metabolism, to preface one's remarks with reference to Garrod. There have been three phases in the discovery of disorders of amino acid metabolism. The first phase lasted from Garrod's work to the chromatographic innovations of Dent and his colleagues. The second phase was a result of the development of these paper chromatographic methods for screening for abnormal 'ninhydrin-positive' metabolites. About 1970, the rate of discovery of new 'ninhydrin-positive' compounds began to lessen and this gave way to the search for 'ninhydrin-negative' metabolites using the newer techniques of gas chromatography and gas chromatography–mass spectrometry. These techniques were responsible for the discovery of the organic acidaemias, secondary to defects of amino acid degradation. This third phase has coincided with an increasing recognition of genetic heterogeneity of inborn errors of metabolism; with the advent of prenatal diagnosis, and the use of pharmacological doses of vitamins in the treatment of specific enzymic defects. The study of inborn errors of amino acid metabolism is now as exciting as it has ever been, and a book dedicated to advances in this field is always of interest.

This book edited by Nyhan is beautifully produced with excellent contributions from many outstanding authorities. It provides, however, an illustration of the problems involved in producing a multi-author text that is balanced and up-to-date. The logistics of obtaining individual chapters from many authors ensures that such a book is out of date before it arrives at the printers. It is useful to compare this book with *Amino Acid Metabolism and its Disorders* by Scriver and Rosenberg (1973). In that volume two distinguished authors covered the whole field themselves, continually revising each chapter, so that the book contains as many current references as possible There is little difference between these two books in the number of references dated later than 1972, although the book by Scriver and Rosenberg was published one year earlier.

A proper balance in a multi-author text is difficult to achieve. In his preface Nyhan refers to Garrod's work on alkaptonuria and albinism. A masterly review on albinism accounts for 11% of the total length of this book, whereas alkaptonuria is not mentioned again. There are two chapters on phenylketonuria and as they overlap it is difficult to see the reason for a second chapter. The two chapters on homocystinuria are, however, useful. The first is by Perry concerning 'classical' homocystinuria and the second by Mudd is an elegant description of the more recently discovered forms, especially those secondary to defects of vitamin B_{12}, and folic acid metabolism.

The lack of balance in this book is also reflected in the description of various methodologies used in clinical and experimental studies. Directions are given for establishing a screening programme for metabolic disease based on a programme in use at the University of California. No mention is made, however, of the Guthrie 'microbioligical inhibition assay', a technique used throughout the world for screening for phenylketonuria. There are unnecessary directions for the chromatography of simple sugars and the diagnosis of mucopolysaccharidoses A chapter on the analysis of radioactive amino acids contains three pages dedicated to the description of fluorescent spectra of liquid scintillators, a list of those available, and recipes for various 'cocktails' for scintillation counting.

This book is beautifully produced but lacks balance, excellent chapters existing cheek by jowl with inadequate or unnecessary material. Its expense is no doubt related to its excellent presentation. Unfortunately, it cannot be recommended as a general text although many individual contributions are of the highest quality.

D. Gompertz

New aspect of old subject

Palaeoeconomy. (Second Volume of Papers in Economic Prehistory. Edited by E. S. Higgs. Pp xiii+244. (Cambridge University Press: London, 1974.) £8.50; $25.00.

THE first volume of *Papers in Economic Prehistory* (edited by E. S. Higgs, 1972) established the theoretical foundation for this major research project; the present volume concentrates on applications of the new methodology to field situations. For newcomers, there is a brief glossary of terms used and a justification of the general approach in a short but provocative review by Higgs and Jarman. They conclude that archaeology should now concern itself with searching for natural laws governing human behaviour, and that the primary adaptation to the environment is in the management of the household; therefore, the 'palaeoeconomic' approach to archaeology should be the most rewarding.

The remaining five papers in the volume are presented to justify this viewpoint. Wilkinson argues that 20th century studies on musk ox behaviour can be extrapolated back to predict the ways in which musk oxen could have been exploited prehistorically, and that these predictions are supported by the archaeological record. Sturdy's analysis of reindeer economies introduces a theme shared by several of the other papers: the difficulty of detecting seasonal mobility (such as transhumance) when archaeological remains recovered from sites of different seasonal activities of the same cultural group may differ as much as remains from quite unrelated cultural groups. Here, analyses of the economic potential of the territory around the archaeological sites may help by providing some indication of seasonal interdependence of areas of summer and winter grazing neither of which alone could provide year-round subsistence. Thus Barker, and Jarman and Webley, working in different parts of Italy, show that patterns of transhumanance recorded in historical times probably had their roots in the seasonal migrations of Palaeolithic hunters and developed throughout the Neolithic as stock were moved between lowlands and highlands for grazing

The two papers on Italian prehistory, and that by Dennell and Webley on Bulgaria, also show that when intensive site surveys and analyses are carried out, the usual cultural and chronological divisions (Palaeolithic, Neolithic, and so on) become rather meaningless. The studies reported here suggest more diversity within cultural periods and also more continuity between cultural periods than has been accepted traditionally.

Appendices include a guide to methods for carrying out site catchment analysis in the field and a discussion by Vita-Finzi of "related territories" and alluvial sediments. There are many maps and figures and these have presumably helped to push the book's price into the range in which libraries will be the chief purchasers.

Barbara Pickersgill

Fowl histology

The Histology of the Fowl. By R. D. Hodges. Pp. xv+648 (Academic: London and New York, November 1974.) £13.50; $35.00.

THE need for a comprehensive work on the histology of the fowl has been apparent for many years. With this volume Dr Hodges has not only met this requirement but has also produced a definitive text for all those engaged in teaching or research on the domestic fowl. In 10 lucidly written and authoritative chapters the author has covered all the systems of the body and has synthesised the widely scattered literature into an eminently readable form. Not content with the descriptions of others, Dr Hodges has himself painstakingly studied the fowl, tissue by tissue, and the resultant descriptions are a distillate of wide reading and critical evaluation by an experience observer.

Each chapter is based mainly on conventional microscopy and is extensively illustrated with photomicrographs and line drawings. Just a few of these fall short of the highest quality and should be replaced in subsequent editions. Electron microscope and histochemical studies are also described, carefully assessed and presented in tabular form for the convenience of the reader. Where controversy exists, as in the mode of formation of the gizzard lining, the author evaluates the evidence and offers the reader his reasoned conclusions.

The descriptions of the reproductive, blood, circulatory, lymphatic and respiratory systems are notable for their clarity and provide clear guidelines in fields where there has been much terminological confusion. They will be particularly useful to those engaged in physiological studies or research in avian diseases.

To many, not the least valuable part of this book will be the extensive lists of references to the avian histological literature which are provided at the end of each chapter. There can be little doubt that this volume will become the standard work on the histology of the fowl.

K. J. Hill

THE PHYSICAL FOUNDATION OF LANGUAGE

Exploration of a hypothesis

R. M. ALLOTT

The hypothesis explored is that, beyond the possibly natural foundation of syntax suggested by Chomsky, the sounds and meanings of words forming a major part of English vocabulary are related Besides survey of experimental material on phonetic symbolism and examination of the adequacy of traditional etymological accounts of the origin of English vocabulary, the main theme of the book is that the natural link between sound and meaning is made apparent in gesture A system of equivalences between component sounds of words and component elements of gestures is presented The claim is that what has been called the 'gestural code' has been cracked (going beyond recent discussion of paralinguistic features and particularly Birdwhistell's analysis of body movements) Evidence of a similar natural relation in other languages is also presented, manifested in striking resemblances between word-sound patterning in many languages for precepts which are sharply and uniformly defined, particularly words for parts of the body, basic features of the environment, colour-words, pronouns and demonstratives.
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Immunology

Lymphocyte Stimulation. By N. R. Ling and J. E. Kay. Completely revised edition. Pp. xii+398. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1975) Dfl.110; $42.50.

LYMPHOCYTE stimulation encompasses most of immunology; in addition, it has long served as a popular and accessable system for studying the control of cell proliferation. Thus the boom in tumour biology and the continuing popularity of immunology assure that this second edition of Ling and Kay's *Lymphocyte Stimulation* will be widely read, if not widely purchased at $42.50. Although I have not read the first edition, it is clear that the second has been largely rewritten. It reviews the properties of T and B lymphocytes and their stimulation by specific antigens and nonspecific mitogens in a thorough and readable fashion.

The first two thirds of the book deal with the descriptive biology of lymphocytes, their subpopulations and properties, methods of culturing them, their *in vitro* responses to soluble antigens, allogeneic cells (mixed lymphocyte reactions) and nonspecific mitogens, (lectins, bacterial products, antibodies to surface antigens), and the properties of activated lymphocytes. Though they present a reasonably up to date review, these chapters are considerably weakened by the omission, or cursory treatment, of various recent and exciting advances in cellular immunology that are relevant to lymphocyte stimulation. Examples of these are the I-region associated antigens (Ia), the physical association of $\beta 2$-microglobulin with the major histocompatibility antigens, the possibility of cooperation between subpopulations of T cells (which may under some circumstances recognise different histocompatability antigens) in the generation of cytotoxic cells, and the general importance of suppressor T cells.

The final third of the book deals with what is known about the molecular mechanisms of lymphocyte activation and the metabolic changes occurring during the activation process. These chapters, which deal almost exclusively with lectin stimulation of T lymphocytes, are thorough and well reasoned, and are the best discussion of this subject that I have seen. They provide an excellent background for anyone attempting to evaluate the most recent evidence for the possible roles of Ca^{2+} and cyclic nucleotides in the early stages of the activation process.

I suspect that the first part of the book will be particularly useful to cell biologists interested in the lymphocyte as a model of induced cell proliferation, and that the latter part will be of most interest to immunologists, who must in the end come to grips with the molecular biology of lymphocyte activation. The extensive bibliographies that follow each chapter should be invaluable to anyone working on and/or writing about lymphocyte responses.

Martin Raff

Combustion

Combustion: The Formation and Emission of Trace Species. By John B. Edwards. Pp. xiii+240. (Ann Arbor Science: Ann Arbor, Michigan, Wiley: Chichester, September 1974.) £10.80.

AN understanding of the genesis of air pollutants in combustion processes is an important and necessary prerequisite to the assessment and engineering of emissions control measures. This book goes some way towards providing a basic text, drawing together pertinent information previously scattered widely throughout the scientific literature. Choosing a predominantly chemical approach, the author has presented in five short chapters a non-mathematical account of premixed flames, secondary combustion processes (thermal reactors, after burners and catalytic reactors), diffusion flames and applications.

The non-mathematical treatment, doubtless adopted with a broad spectrum of readers in mind, has almost entirely precluded any discussion in depth and so the book fails to reach the level of a basic text and reference volume that is claimed of it; indeed a certain superficiality results in places. It would not satisfy the requirements of a British graduate course in science or engineering without considerable supplementation. On the other hand, it should serve admirably as an introductory text and as a guide for those whose primary concern is with other aspects of the air pollution problem.

Although the author admits to only minimal consideration of the detail of specific applications it is unfortunate that the final chapter is little more than an excellent selective bibliography of nearly 300 references. Treatment of one or two applications, even if only for illustrative purposes, would have given the reader an idea of the complex interactions between the chemical, engineering, economic and legal aspects of the emissions problem, thus adding context to the book.

Nevertheless, this useful volume fills a definite gap in the literature.

N. H. Pratt

Small points

The Mitochondria of Microorganisms
By David Lloyd. Pp. xii+53
(Academic: London and New York,
January 1975.) £13.60, $36.00.

MITOCHONDRIA have been the subject o
study and speculation since around
1890 but until a satisfactory method o
isolation was devised by Hogeboom
Schneider and Palade in 1948, little pro
gress was made. During the followin
15 years immense effort was devoted t
the study of oxidative phosphorylatio
in animal mitochondria but relativel
little attention was given to the mito
chondria of yeasts, moulds and pro
tozoa. The isolation of mitochondria
DNA in 1964 and the demonstratio
that this DNA had genetic independ
ence made eukaryotic microorganism
attractive as tools for the study of mito
chondrial function.

Until now, however, there has bee
no adequate text book for those enter
ing the field so that in writing this book
David Lloyd has no competitors. Thi
is not an easy book to read but it
accurate, up to date and comprehen
sive; indeed, the difficulty presented t
the reader is occasioned largely as
result of the determination of th
author to leave no unexplored corner
Most of the significant work on mito
chondria has been done with organelle
from animals, yeasts or neurospora, bu
other lesser known organisms ar
covered so thoroughly in the text tha
at times it is difficult to distinguish th
significant from the somewhat trivia
This is, however, a minor criticism
a very worthwhile text book. It
symptomatic of the changed outlook i
the field that relatively little attentio
is devoted to oxidative phosphorylatio
and that the main emphasis is on bi
genesis and on genetics of mitocho
dria. But there is an excellent a
very necessary review of the need f
good, clean, undamaged mitochondri
preparations if meaningful results a
to be obtained.

It is no easy matter when publishi
a major textbook to ensure that it
up-to-date. Dr Lloyd is to be congrat
lated, therefore, on his success in t
respect. The book is divided into thr
parts: first, structure and functio
second, biogenesis; and finally, evol
tion; but it is not always easy, havi
read the text, to find again some pa
ticular bit of information which m
be wanted. This is my only maj
criticism of a first class text book. Mo
cross references within the text and
more comprehensive index would a
greatly to the ease with which the va
amount of information collected
Lloyd could be used by his colleagu
and by future students. **T. S. Wo**

obituary

Ove Frydenberg, known for his work in experimental population genetics of *Drosophila* and, in recent years, of protein variation in fishes, died in Denmark on April 7, at the age of 45.

After studying genetics and biometry at the University of Copenhagen, where he earned his Magister degree in 1955, he joined the staff of the Genetics Department there and lectured on population genetics and evolution. He also lectured on human genetics at the Dental School and served as consultant and collaborator for the barley genetics research group of the Danish Atomic Energy Commission. He spent two years in Brazil and one in the United States engaged in research and study. In 1964 he received his Dr Phil. from the University of Copenhagen. During this period his researches were diverse, including *Drosophila* systematics and genetic studies on barley, fish and humans. Also he was clearly one of the first to engage in a critical dissection of the selection process, using *Drosophila* as a tool. In 1967 he was given the mission of establishing a genetics institute at the University of Aarhus where he was appointed Professor and Chairman His eight years of leadership resulted in a vigorous teaching and research organisation of international stature. During this last phase of his career he became an important figure in the Scandinavian science community. He wrote textbooks for Danish schools and popular articles on genetics. He was a board member of the Scandinavian Genetics Society and member of the Advisory Board of the Danish Atomic Energy Commission, Danish National Scientific Research Council and Royal Academy of Science and Letters. He completed a full and productive career during his short life.

Max Rudolf Lemberg, who died in Sydney on April 10, will be remembered for his contributions to pyrrole chemistry and biochemistry, as a pioneer of Australian biochemistry and as a Quaker.

Born in Breslau in 1896, his first postdoctoral work was in heterocyclic chemistry with Heinrich Biltz of Breslau University. After a short period finding that he did not like industry, he accepted Freudenberg's invitation in 1925 to work on natural products at Heidelberg University. There he acquired his special skills in bile pigment chemistry, with several prized victories over the Hans Fischer school which then dominated tetrapyrrolic chemistry. He demonstrated that the chromoproteins of the red and blue algae bear a bile pigment chromophore by isolating crystalline mesobiliverdin ester; he showed that oocyan and uteroverdin were both biliverdin and that the 'green haemin ester' of Warbarg and Negelein was not a haemin but biliverdin ferrichloride ester In 1933 he was forced to leave Germany, and went to Cambridge where he had previously come under the influence of Hopkins, Barcroft and particularly Keilin who had much influence on his future interests. From Cambridge he accepted in 1935 an appointment as Director of the Biochemical Laboratories at the Institute of Medical Research in the Royal North Shore Hospital of Sydney. With the help of J. W. Legge, among other colleagues, he elaborated the sequence of initial oxidative ring opening of the tetrapyrrolic macrocycle while the iron was still present, followed by removal of the iron to form biliverdin which in turn could be reduced by a specific liver reductase to bilirubin. This is today the accepted pathway of haem catabolism to bile pigments. Lemberg's next field was another green haem, the prosthetic group of cytochrome oxidase. He first attacked this through a study, with the late J. E. Falk, of the effect of side chains and their order on the spectra of porphyrin and the corresponding haems and haemochromes. This was followed by painstaking development of methods of isolating the rather labile porphyrin *a* with identification of several side chains. He then worked until his retirement on the mechanism of the reaction of cytochrome oxidase with oxygen, including the spectroscopic nature of the first components with oxygen and the accompanying confirmational changes. Lemberg's study of the literature was meticulous and systematic and this appears in his two books. *Haematin Compounds and Bile Pigments* by Lemberg and Legge has been the standard work on the subject since its appearance in 1949. *The Cytochromes* by Lemberg and Barrett appeared in 1973 and critically reviews the literature from 1949 to 1972. Of many public recognitions Lemberg prized most his election to the Royal Society in 1952 and his appointment in 1956 as Professor Emeritus of Heidelberg University. He gave time generously to scientific societies and national committee work and was a foundation member of the Australian Academy of Sciences and of the Australian Biochemical Society.

announcements

Awards

The National Academy of Sciences has announced the election of the following as new members in recognition of their distinguished and continuing achievements in original research: S. A. Adler, H. N. Andrews, C. E. Ballou, G. S. Becker, E. P. Benditt, B. J. L. Berry, H. S. Bloch, B. S. Blumberg, M. Boudart, K. E. Boulding, F. A. Bovey, R. O. Brady, Jr, J. R. Brobeck, C. M. Brooks, J. J. Burns, G. W. Burton, K. M. Case, B. Chalmers, M. W. Chase, M. J. Cohen, Z. A. Cohn, J. P. Collman, L. N. Cooper, G. M. Cox, E. C. Creutz, F. A. de Laguna, A. H. Doermann, P. Elias, W. G. Ernst, H. Federer, E. Feenberg, G. Feher, H. Frauenfelder, D. Fultz, P. R. Garabedian, R. G. Gordon, C. W. Gottschalk, H. Z. Griliches, R. E. Gross, F. M. Haurowitz, W. Henle, R. L. Hill, R. H. Holm, D. M. Horstmann, L. M. Hurvich, D. Jameson, R. W. Kates, K. I. Kellermann, J. C. Kiefer, D. E. Knuth, A. H. Lachenbruch, E. N. Lorenz, E. Margoliash, M. V. Mathews, M. Mead, E. T. Mertz, M. F. Morales, J. N. Morgan, E. W. Mueller, D. B. Mumford, J. E. Myers, A. A. Penzias, V. R. Potter, P. B. Price, Jr, C. F. Quate, R. Radner, W. P. Rowe, H. A.

Schneiderman, M. Schwartz, C. G. Shull, P. Siekevitz, H. E. Simmons, Jr, E. H. Spicer, C. M. Stein, D. F. Steiner, G J. Stigler, G. Streisinger, I. Tamm, G. Weber, J C. Wheatley, H. C. White, R. H. Whittaker, R. S. Yalow.

The National Academy of Sciences has announced the election of the following as foreign associates: W. Beermann, C. de Duve, R. Hill, N. K. Jerne, K. Kodaira, D Lal, E. J. Opik, A. E. Ringwood, M. C. Mendes da Rocha, Sir Martin Ryle, E. N. Sokolov, G. Wilkinson.

The following awards have been made by the British Institute of Radiology: **Barclay Medal** to **F. Doyle** (pathogenisis and radiological appearances of metabolic bone disease); **Barclay Prize** to **F. Starer; Röentgen Prize** to **H. Hewitt** (comparative survival of irradiated tumour cells).

Appointment

G. W. Fenton has been appointed to the chair of mental health at the Queen's University, Belfast.

International meetings

July 20–28, **Regulation of metabolic pathways,** Bogota, Columbia (Dr C. Corredor, Bioquimica, Facultad de Medicina, Universidad del Valle Apartado Aereo 2188, Cali, Colombia).

July 22–25, **Mechanisms of protein-hormone action,** Fort Collins, Colorado (Dr Lloyd Faulkner, Chairman, Local Committee, SSr, Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80521).

July 22–25, **Cryogenic engineering conference,** Kingston, Ontario (L. Kenneth Armstrong, National Bureau of Standards, Boulder, Colorado 80302).

July 26–27, **Active intermediates: formation, toxicity and inactivation,** Turku, Finland (Dr Harri Vainio, Department of Physiology, University of Turku, Kiinamyllynkatu 10, SF-20520 Turku 52, Finland).

July 28–30, **Heavy metals as environmental hazards,** Bristol (Miss M. V. Auguste, The Chemical Society, Burlington House, London W1V 0BN, UK).

July 28–30, **Conduction and breakdown of dielectric liquids,** Delft (Dr J. M. Goldberg, Chairman, Organising Committee, Department of Applied Physics, Delft University of Technology, Lorentzweg No. 1, Delft, The Netherlands).

Person to Person

London accommodation. American professor and family desire 3 bedroom apartment or small home within 20 min of Imperial Cancer Research Fund Laboratories for 1 year, beginning July or September, 1975. Prefer Hampstead Heath area (Dr I. Diamond, Department of Neurology, University of California, San Francisco, California 94143)

Toronto house. University of Toronto scientist seeks a responsible family for his furnished house while he is on sabbatical leave. Four bedrooms, convenient schools and public transportation. Available from September 1, 1975 to August 31, 1976 (Dr A. Goodridge, C. H Best Institute, 112 College Street, Toronto, Ontario, Canada M5G 1L6).

Sea sticklebacks. Wanted: shipment of live sea sticklebacks *Spinachia vulgaris* For details: Dr G. E. Moodie, Department of Biology, University of Winnipeg, Winnipeg, Manitoba, Canada R3B 2E99.

There will be no charge for this service Send items (not more than 60 words) to Robbie Vickers at the London office The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted No commercial transactions

Reports and publications

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The Development of Radio. By Ian Trethowan. (BBC Lunch-Time Lectures, Ninth Series, No. 4.) Pp. 15. (London BBC, 1975.) [103

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Professional Conduct Guidance for Chemists. Pp. 32. (London. Royal Institute of Chemistry, 1975.) [103

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An Illustrated Guide to Aquatic and Water-Borne Hyphomycetes (Fungi Imperfecti) with Notes on Their Biology By C. T Ingold (Scientific Publication No. 30) Pp. 96 (Ambleside, Westmorland Freshwater Biological Association, 1975.) £1 [133

Centre for Overseas Pest Research Tropical Pest Bulletin No. 4 Seasonal Distribution and Migrations of *Agrotis ipsilon* (Hufnagel) (Lepidoptera Noctuidae). By P. Onyango Odiyo Pp 26 75p net. Anti-Locust Memoir No. 12 An Outbreak of the Australian Plague Locust, *Chortoicetes terminifera* (Walk), During 1966–67 and the Influence of Weather on Swarm Flight By M Casimir and R C. Bament. Pp 27. 65p net (London Centre for Overseas Pest Research, College House, Wrights Lane, W8, 1974 and 1975) [133

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Loughborough University of Technology Report to the University Court, 1973/1974 Pp 158. (Loughborough. University of Technology, 1975.) [143

The Hydrogen Bond and Other Intermolecular Forces. By J. Clare Speakman. (Monographs for Teachers, No 27) Pp. iv + 33 (London The Chemical Society, 1975.) 60p. [173

Commercial Assessment of Recently Introduced Potato Varieties—Report on Trials and Surveys 1974. Pp 25 (London Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) *gratis.* [173.

The Wellcome Trust 1972/1974—Tenth Report. Pp. 124. (London. The Wellcome Trust, 1 Park Square West, 1975) [173

Bulletin of the British Museum (Natural History). Entomology. Vol 31, No 7. A Revision of the Ant Genus *Leptogenys* Roger (Hymenoptera: Formicidae) in the Ethiopian Region, with a Review of the Malagasy Species By B Bolton Pp 235–305. (London: British Museum (Natural History), 1975.) £4.05. [173

The Living Coelacanth (Zoology Leaflet No. 10) Pp 8 (London British Museum (Natural History), 1975) 7p. [203

Other countries

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Studies and Reports in Hydrology. No 5 Discharge of Selected Rivers of the World. Vol. 3, Part 2 Mean Monthly and Extreme Discharges (1969–1972). Pp. 124. No. 17: Methods for Water Balance Computations An International Guide for Research and Practice. Edited by A. A. Sokolov and T. G Chapman. Pp. 127 (Paris. Unesco Press, London HMSO, 1974) [173

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nature

May 29, 1975

He wants your opinion

IT has been a busy week for Lord Crowther-Hunt, Minister for Higher Education at the Department of Education and Science, who has delivered three major speeches on the subject of priorities within the higher-education sector. These, coupled with Mr Prentice's recent appearance before a House of Commons Select Committee, go some way towards reassuring those in higher education that the department, which has kept a distinctly low profile in this field for a long time, has not forgotten about them entirely. And the generally thoughtful nature of these contributions, positively inviting dialogue, deserves an intelligent response from the intellectual community and from employers.

The problem confronting educational planners is this. At present there are about 460,000 full-time students in universities and in polytechnics and colleges in England and Wales. There is still a commitment to raise this figure to 640,000 by 1981. But this sort of expansion could not be carried out against a background of economic stagnation unless the government were to devote what growth money it had to higher education, to the detriment of schools, hospitals and so on. This it clearly will not do; Lord Crowther-Hunt does at least go through the motions of musing whether there is a strong relationship between investment in higher education and long term economic growth, but concludes only that "it would be asking too much for a reasonable man to believe that a higher quality labour force does not have a higher potential than a lower quality one, while recognising at the same time that quality in this context has many dimensions". Hardly a strong case for Mr Prentice to argue in Cabinet for preferential treatment for higher education to get us out of our economic hole. Thus the recent Budget cuts of £1,000 million in public expenditure planned for 1976/7 mean substantial cuts in education and science, and instead of a growth rate of 2.7% in that year, there will only be room for about 1% more.

Inevitably, one must then ask whether expansion to 640,000 makes sense, and if it does, what sort of education this means for the swollen numbers. The answer to the first question is almost certainly that the target will not be reached, although obviously the government would like not to have to be responsible for taking that decision itself; it may well be let off the hook by the continuation of the trend amongst the young to forego higher education voluntarily. But even if targets are lowered, it is inescapable that the atmosphere within the higher education world will have to change. Lord Crowther-Hunt dropped several hints of things to come:

- Student/staff ratios are bound to rise from their present value in universities of 8.4 : 1. A figure of 10 : 1 or even 11 : 1 was mentioned—from which it could be inferred that present staffs would spend more time teaching, rather than that new staff would be recruited.
- Manpower planning will be conducted in an attempt to respond to the perceived future needs of employers.
- Efforts will be made to make courses more relevant to the needs of the country.
- Universities and polytechnics will be urged to collaborate more.
- The balance between teaching and research will have to be looked at.

Some of this can be welcomed, particularly any efforts within the manpower planning field to understand how the young decide what to study—and whether they are open to persuasion to change their mind. The worry must always be whether the planners' crystal balls perform any better than randomly in predicting needs five or ten years hence. Any positive intervention at present would presumably be devoted to restocking chemistry, and to a lesser extent, physics departments; it would be unlikely to be urging yet more students to think of medicine as a career. Yet how much confidence can we really have that by responding to present imbalances we shall create future prosperity? Maybe the chemical industry would profit more from an influx of mathematicians and the medically-trained than by continuous recruitment of chemists, and maybe this is unknowable before the fact.

Similar concern must also be expressed that 'relevance to the nation's needs' can be pushed much further without producing dreary courses of study away from which bright students will flee. Lord Crowther-Hunt's contribution to the debate here is less than worthy—"many educationists will disagree [with relevance] but they are surely going to find it very difficult indeed to argue for the contrary principle, namely 'irrelevance' ". Many besides educationists will find such stark categorisation unhelpful. The contrary principle to which many devote their teaching career is intellectual agility and flexibility and is surely as much to be sought after now as ever.

There has as yet been scarcely a murmur from the academic community at the increase in productivity called for if student/staff ratios are to rise. But there is one quite serious problem which deserves early airing. The higher education world is not like some vast nineteenth-century construction industry where the foreman/navvy ratio could be juggled at will by hiring and firing. For better or worse almost all staff have tenure. Further, staff tend to come in thousands of little cells called departments, each with its own proud independence. A smooth change in student/staff ratio at the macroscopic level can produce some hideous anomalies at the microscopic level.

Lord Crowther-Hunt has done the higher-education community a service by giving early public notice of change. The community can help mould its future by an intelligent response. ☐

A headache for US industry

On April 28, 1971, the Occupational Safety and Health Act came into effect in the United States, establishing a new agency—the Occupational Safety and Health Administration (OSHA) in the Department of Labor—with a mandate to enforce health and safety standards for America's 60 million workers. As expected, the act has turned out to be a huge headache to enforce and administer, not only because of the enormous costs to industry in altering its technology and work practices to comply with federal standards, but also because the act does not diminish, but raises, discontent about conditions in the workplace. It exposes the sores of industrial practices and labour union conservatism and raises the profound question of what research in occupational health should be directed towards. Wil Lepkowski reports.

NEXT month, OSHA will begin a series of public hearings on its proposed noise standard of 90 decibels which will be both long and costly. Dow Chemical Corporation alone has asked for five days to present its case against that proposal. Consumer and labour groups, on the other hand, will argue for the figure of 85 dB being pressed for by the Environmental Protection Agency (EPA). (The EPA's standards inevitably are more stringent than OSHA's, on the unspoken assumption that the workplace is inherently more dangerous than the outdoors).

The case against noise will be argued in the context of hearing loss, but another aspect is creeping into the acoustic consciousness: frequency. Psychophysicists are finding that various frequencies can exert subtle but profound effects on the human brain and nervous system. If the 90dB level is adopted (and the EPA will fight its sister agency OSHA for the 85 dB standard to be established), it will cost industry $30,000 million to comply, according to a study by the engineering consultant company Bolt, Beranek and Newman. If research is able to correlate frequencies with, say, the accident rate or the vague condition known as *ennui* then occupational safety and health could reach another level of complication involving design of machinery and the mind–body–environment interaction in the workplace.

Consider stress, for example. The research arm of OSHA, the National Institute of Occupational Safety and Health (NIOSH), recently published proceedings of a conference on behavioural toxicology which raised questions about the role of stress in job safety, health and performance. OSHA is nowhere near developing standards for reducing stress on the job—unless one regards noise and heat as stress inducers. But OSHA's standards director Daniel Boyd agrees that stress is an obvious problem in the workplace which is related to development of chronic disease, and that standards will eventually govern work practices that produce stress. The question, however, is how one separates workplace stress from stresses of the home, family, and environment. At what point does occupational health spill over into community health?

Cancer is often considered to be a disease induced in the workplace, but heart disease is not. Why? Because carcinogens are knowns to be present in the workplace, whereas non-psychological causes of heart disease are harder to spot. Thus, heart disease is considered a community health problem, whereas cancer is increasingly looked on as a job-related disease.

The new NIOSH Director, John Finklea, will have plenty of housework to do before he ventures out with his first indictment. The agency's entire research programme is ripe for reorganisation, for example. "There's no direction to the research they do there," declares Nicholas Ashford who is completing a large study of the occupational safety and health for the Massachusetts Institute of Technology Center for Policy Alternatives. "There are no deliberate attempts to formulate any kind of research policy based on real problems in the workplace," he says. "They don't bring input from labour into the design of their research projects. What they have is a lot of old-time researchers who are accustomed to pursuing their own interests and going their own way without regard to the timeliness of their work".

Sheldon Samuels, director of health, safety, and environmental affairs for the Industrial Union Department of the AFL-CIO, is even harsher. "They have a lot of dead wood and meaningless programmes there," he says. "Their hazard evaluation programme is a total failure. The evaluations they do are meaningless. They don't really evaluate present and future hazards. All they do is measure how close companies come to the threshold limit values. And when they get important information they sit on it without al ing anyone."

Finklea is keenly aware of the cr cisms. One of his plans is to reduce 10% all newly induced cancers in workplace. The strategy is unclear a as the forward plan for NIOSH sta "the full impact may not be evident approximately 20 years because of disease latency period". What will c tainly be involved is coordination w several agencies that deal with carci genesis, as well as a much stepped cancer epidemiology programme. I example, the National Center Health Statistics has no data on dise broken down by occupation. That have to change.

Just as rising water finds ev nook and cranny in a tidal cave, OSHA extends into every occupat in the United States. In the operat theatre, studies have shown that ana thetic gases are the cause of bi defects in the children of nurses posed to those gases and even in children of male physicians simila exposed. OSHA is satisfied, howev that the medical profession is doing adequate job of regulating its o operating room environment now t the danger is known.

It is even conceivable that OS could regulate the methodology genetic research if the scientists the selves do not succeed in drafting a of procedures that prevent the spre of potentially hazardous organisms i the atmosphere. The subject was rai by a Washington lawyer, Har Green, during the meeting on gene transplantation organised by the Na onal Academy of Sciences at Asilom California.

A lawyer from the University Indiana, Roger Dworkin, warned t if scientists did not regulate that search, OSHA could move in and d for them.

The individual most responsible the direction that occupational hea research takes at a national level John Finklea. A South Carolina p diatrician, he has a penchant for c troversy as well as skill at diplom and could be a much-needed antid to his predecessor Marcus Key, w preferred diplomacy at all times. head of the National Environmen Research Center under the EP Finklea was in the thick of the deb over sulphate pollution from pow plant chimneys and from the cataly converters in automobiles. Suffice it say that Finklea's position as an opp ent of tall chimneys and the cataly converter hastened his departure fr the EPA.

Finklea has the backing of b environmental and labour groups, industry is cautious. "It's true Fink doesn't shy away from controvers says Kenneth Nelson, vice-president for environmental affairs at American Smelting and Refining Company, which was stung by NIOSH's recommendation of a no detectable limit standard for arsenic. OSHA went along with the recommendation and issued it as a proposed standard in March. "His pronouncements on sulphates at EPA were quite devastating. There's absolutely no evidence that they are dangerous in environmental concentrations. But suddenly his position became gospel. I don't want to prejudge him, but this is the kind of thing we don't want to see him do at NIOSH."

The most scaring thing NIOSH deals with is the fear that an 'epidemic' of industrial cancer may be beginning which is several years away from hitting with full force. There's no apparent way to prevent such an event from happening once the cancer process has begun. "We're talking about problems we will have 60 years from now," says Finklea. "That is something we've never faced in NIOSH."

The vinyl chloride episode stunned industry into recognising some of the more frightening consequences of exposure to toxic substances. It was also a huge embarrassment to all the government agencies which oversee environmental health. NIOSH was shocked and embarrassed, but not as acutely as the National Cancer Institute (NCI). NIOSH, after all, could plead scarcity of funds and manpower with its overall budget of $700 million. The NCI had no such let-out. Critics said correctly that it was guilty of misplaced priorities. Now it is trying to catch up.

Since that episode, the institute has stepped up its cancer bioassay programme to intensify its relevance to occupational exposure to carcinogens. It now issues so-called 'cancer alerts' to an inter-agency panel whenever environmental and epidemiological researchers at the National Cancer Institute come across data showing links between chemicals in production and the cancer process. The latest such alert was issued for trichloroethylene, triggering action by NIOSH's hazard surveillance teams to assess the extent of the problem throughout industry.

The NCI knows it needs much more information about carcinogenesis on the personal level—that is, on the level at which the work environment affects cancer susceptibility and, in fact, prevention. Its newly emphasised cancer control programme will begin studies on cancer control in the workplace. And the environmental carcinogenesis programme under Dr Herman Kraybill, is gradually working out written agreements with companies that will give NCI researchers access to the medical workers exposed to chemicals suspected of being carcinogenic.

Finklea: not afraid of controversy

Industry itself has made a quantum leap into responsibility, not only by opening up its records—unprecedented in the past—and broadening its medical examination of workers to a score of metabolic parameters but also through formation of an inter-company toxicology research programme to give early warning to companies about impending problems. The project is new and requires the working out of some legal difficulties over transfer of what once was considered privileged information. But now worker health is taking precedence over industrial secrecy.

"What we're seeing now," says Kraybill, "is industry moving slowly towards closed systems in its chemical production processes. The big companies can do this. But it's the smaller companies that are causing many of the problems". The small companies do indeed present difficulties. In most cases they exist in small towns where the work force is small and has few other job opportunities. Profits are not substantial, and work and production practices are sloppy. In one rural town in Maryland a small company was found to be poisoning the entire community through the dumping of chemical wastes in an adjoining stream. Research offers no solution to this kind of situation. And even now, it has not been possible to find the customary one-to-one ratio between the offending agent and the mortality in that Maryland village.

Although occupational health researchers see no broadly encompassing methodology that would substitute for the chemical by chemical assessment of health hazards, they are agreed that a single standard test protocol for bioassay can be developed. The process is somewhat akin to working out an international treaty: a text is drafted and redrafted until each faction makes the compromises necessary for agreement. The National Cancer Institute is being looked on by the toxicology profession as a high arbiter in the resolution of controversy. It is not a regulatory agency but is the focus for most frontier regions in cancer research and so can be depended on to harness the information needed to present a consensus.

Finklea realises the need for a generalised research theory of occupational health but sees none on the horizon. What he would like to see at the very least is NIOSH run as a single 'system', flowing from workplace problems, to applied and basic research needed to understand it, to the production of a criteria document or OSHA recommendation. As such, he is as much directing policy research as a series of projects. Each criteria document alone now costs close to $1 million in contractor costs, far too high and counterproductive. And OSHA very often rejects the recommendations set out in them. In the science policy sense, NIOSH, by focusing just on the human problem, does seem to synthesise in one place the many aspects contained in Francis Bacon's dictum to scientists: " . . . that they consider what are the true ends of knowledge, and that they seek it not either for pleasure of mind, or for contention, of for superiority to others . . . but for the benefit and use of life; and that they perfect and govern it in charity." □

international news

Report wants controls on bacteria work

UNLIKE radioactive substances, and a few animal pathogens such as foot and mouth virus, work on pathogenic microorganisms in the United Kingdom has been subject to few statutory controls. Laboratories working with many viruses and bacteria pathogenic to humans and animals are not required to register their work and such codes of practice that do exist and are followed are voluntary and drawn up by the workers themselves in self-defence.

But opportunities for work with 70 pathogenic bacteria, viruses and other microorganisms will be severely curtailed in the future, according to a report published last week by a working party under the chairmanship of George Godber (former Chief Medical Officer of Health at the Department of Health and Social Security, DHSS) which was set up to investigate the laboratory use of dangerous pathogens following the escape of smallpox virus from the London School of Hygiene and Tropical Medicine in 1973.

The first task of the working party was to draw up a list of laboratories working on a check list of 66 pathogens considered particularly dangerous. A questionnaire sent to all biological laboratories in the country, including those in universities, hospitals, the public health service and industry, identified 595 laboratories holding one or more of the pathogens listed. This says the report is a much larger number than expected.

The most widely held organism was *Salmonella typhi* (held by 322 laboratories) followed by *Mycobacterium tuberculosis* (289 laboratories), *Vibrio cholerae* (194 laboratories) and *Brucella* (179 laboratories). The working party was particularly concerned that *S. typhi* was one of the organisms used for routine testing of disinfectants and recommends that the relevant British Standard be amended to permit another suitable organism such as *Escherichia coli* to be used. It also recommended that the practice of using pathogenic strains of *M. tuberculosis* for teaching should be discontinued.

Its main recommendations were that 30 of the most dangerous organisms (including herpes B virus Lassa fever virus, foot and mouth virus, rabies virus and smallpox virus) should be designated category A organisms and should only be studied under the most stringent conditions. Control of their possession and use should be exercised by some central body. Category B organisms (including many of the human and animal bacterial pathogens) should only be studied in laboratories under the control of suitably qualified staff following codes of practice yet to be drawn up but similar in essence to guidelines contained in *Safety in Pathology Laboratories* and *The Prevention of Laboratory Acquired Infection* (DHSS).

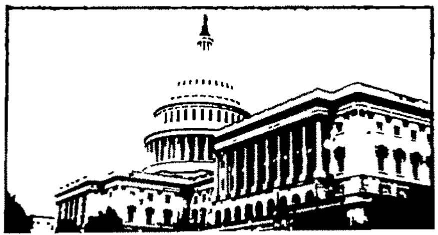
Category A Pathogens	*No of establishments*
Herpes B virus	4
Lassa Fever virus	1
Marburg virus	1
Rabies virus	13
Smallpox virus	19
African Horsesickness virus	1
African Swine Fever virus	3
Bluetongue virus	1
Equine Encephalomyelites group	29
Foot & Mouth Disease virus	2
Fowl Plague viruses	24
Japanese B virus	4
Lumpyskin Disease virus	1
Newcastle Disease virus	63
Rift Valley Fever virus	6
Rinderpest virus	2
Saint Louis virus	2
Sheep Pox virus	5
Swine Fever virus	2
Teschen Disease virus	6
SVD virus	1
Vesicular Exanthema virus	1
Vesicular Stomatitis virus	14
Wesselsbron virus	2
Francisella tularensis	3
Histoplasma farciminosum	0
Theileria, all species	8
Trypanosoma cruzi	16
Trypanosoma equiperdum	6
Trypanosoma vivax	12
Category B Pathogens	
Arboviruses (human)	45
Arboviruses (animal	35
Aujesky's Disease virus	21
Bat rhabdovirus	6
Pustular Dermatitis virus	24
Duck Plague virus	7
Equine Anaemia virus	2
Equine Arteritis virus	1
Bovine Rhinotracheitis virus	24
Kyanasur Forest Disease virus	3
Murine rhabdovirus	1
Serum Hepatitis virus	98
Gastroenteritis virus (pigs)	11
Yellow Fever virus	3
Bacillus anthracis	110
Brucella, all species	179
Clostridium botulinum	52
Leptospira, all species	41
Loefflerella mallei	17
Mycobacterium leprae	5
M. tuberculosis	289
Pseudomonas pseudomallei	29
Salmonella typhi	322
Vibrio cholerae	194
Yersinia pestis	29
Rickettsia, all species	3
Coxiella burnettii	23
Chlamydia psittaci	28
Babesia, all species	17
Plasmodium, all species	16
M. mycoides var mycoides	19
M. mycoides subsp. capri	16
M. agalactiae subsp. agalactiae	17
Coccidioides immitis	2
Enzootic Bovine Leucosis agent	0

The recommendations regarding category A pathogens involve the establishment of a Dangerous Pathogens Advisory Group (to be chaired by Professor R. A. Shooter) which would advise on the licensing of individual laboratories and conditions under which work should be done. Although this system would be voluntary at first, it will eventually require statutory controls. Complete confidentiality would be maintained. The import of category A pathogens into the country should also be strictly controlled and should only be allowed when the work to be done is of "overriding importance". Detailed recommendations for dealing with category A pathogens are given in the report. Implementations of these recommendations will require in most cases completely new laboratory buildings.

- That the total number of laboratories holding pathogens in categories A and B should be reduced by ceasing the practice in any laboratory which cannot show good reason for continuing it.
- When work involves any special risk to the public a decision on whether work should proceed should be made outside the laboratory.
- General guidance on safety in laboratories should be drawn up and applied.
- International standards for the control of dangerous pathogens should be drawn up.
- No pathogen should be handled in a laboratory except under the supervision of suitable qualified staff.
- The categorisation of dangerous pathogens included in the report should be kept under review and modified as necessary in the light of future discussions and experience.
- A safety officer should be appointed in any laboratory holding dangerous pathogens. □

Washington seen

by Colin Norman



In a report which must surely rate as one of the federal government's most candid assessments of the troubles afflicting nuclear power in the United States, a task force of the Energy Research and Development Administration (ERDA) concluded last week that the nuclear industry faces a stunted future unless public concern over reactor safety, safeguards against the theft of nuclear material and waste disposal are soon cleared up. Moreover, the report also notes that shortages of uranium could restrict the growth of nuclear power after the mid-1990s, that spent fuel is piling up at reactor sites to such an extent that some reactors may soon have to be shut down, and that the government may have to share the financial burden of building new uranium enrichment plants because the nuclear industry is unlikely to raise sufficient capital on its own.

Although those problems are not new, the task force represents a welcome break from many previous government pronouncements on nuclear power, which have mostly followed the line that the problems have been exaggerated by a group of eccentrics who do not know what they are talking about. This report, however, accepts that the "fears of the lay public and its decision-makers are supported by a fair segment of the scientific community—many of whom otherwise support the use of nuclear reactors for the generation of electric power".

On the question of uranium resources, the report notes that unless either plutonium is extracted from spent fuel and recycled as a fuel for light water reactors, or the breeder reactor is brought on line as soon as possible, there is only enough uranium in the United States to guarantee a lifetime's fuel for a limited number of reactors. The task force therefore urges the Nuclear Regulatory Commission (NRC)—which has responsibility for regulating the nuclear industry—to make up its collective mind as soon as possible on whether or not plutonium recycling should be allowed. But, between the writing and publication of the report, the NRC announced that it probably will not make a decision before 1978 on plutonium recycling. The nuclear industry is therefore faced with great uncertainties about how long the fuel for light water reactors will last.

But the NRC's delay in ruling on plutonium recycling has other consequences. Until the decision is finally made, the NRC will not issue a licence for operation of any fuel reprocessing plants, which means that spent reactor fuel will continue to be stored at reactor sites for several more years. But the task force report notes that 10 reactors do not have any more room for spent fuel storage, and many others will soon face the same problem. The "industry should be advised that additional spent fuel storage capacity is required and that ERDA regards this as an industry responsibility and has no plans for providing such storage", the task force suggests.

On the question of waste disposal, the task force notes that the problems "are tied almost completely to whether or not spent fuel is processed", so that, too, hangs on the plutonium recycling decision. In any case, no method for permanent disposal of radioactive wastes has yet been demonstrated, and the report suggests that problems in finding and demonstrating such a method "together with the lack of decision on the part of the government [have] in fact come close to making waste management the pacing item of the nuclear fuel cycle".

Nevertheless, the task force believes that the problems can be overcome, but that "public understanding and acceptance are essential to the future effective and timely use of fission energy as a source of electric power". The task force therefore calls for "candour and objectivity on the part of the government in dealing with the public". Its own report is a good start.

● Following a number of recent reports in the medical literature that aspirin may help prevent blood clots from forming, the National Heart and Lung Institute has launched a massive clinical study to see whether the drug offers any protection against heart attacks.

During the next year, about 4,200 people aged between 30 and 69 who have previously suffered an acute heart attack will be enrolled in the study, which will be carried out by 30 hospitals and research centres around the United States. Half the volunteers will each receive 1 g of aspirin a day (equivalent to three regular tablets) for three years, while the rest will be given placebos.

The chief objective will be to see whether there is any difference in total mortality between the two groups, and investigators will also be looking for such factors as differences in the incidence of non-fatal heart attacks and strokes between the two groups. According to Dr William Friedewald, the study director, about 85% of those who now survive one heart attack eventually die from a second heart attack or from other cardiovascular problems.

Volunteers will be given quarterly medical checks while they are taking the drug and more extensive annual checks during a three-year follow-up period. A committee at the Heart and Lung Institute will periodically monitor the study and, unless adverse drug effects show up, the clinical phase is expected to be completed in September 1979.

Aspirin may help guard against heart attacks because it hinders the clumping together of blood platelets, which is believed to be a crucial early event in the formation of arterial blood clots.

● The failure last week of the final stage of a Titan III-C rocket during the launch of a pair of military communications satellites is not expected to affect future space science missions, according to NASA officials. At a meeting held on May 21, the day after the mishap occurred, it was decided that the Viking Mars Lander mission should go ahead in July as planned, even though it will be launched by a rocket whose first three stages are similar to those of the Titan III-C.

Officials of NASA are still not certain what caused the failure, however. Control over the final stage was lost before its engines fired, it began to roll helplessly and eventually burned up on re-entry into the Earth's atmosphere.

The mission, which cost $57 million, was to have completed a global military communications network consisting of four satellites. The first two were launched in 1973 into stationary orbits over the Atlantic and Pacific, and last week's launch should have placed the other two over the Indian Ocean and the Pacific.

● Virtually unnoted by the general press, or by Congress, two key government positions affecting biomedical research were filled earlier this month. Dr Donald Fredrickson, President of the Institute of Medicine (part of the National Academy of Sciences) was appointed Director of the National Institutes of Health, and Dr Theodore Cooper, a former Director of the National Heart and Lung Institute, was appointed Assistant Secretary for Health—the top health job in the federal government. Both appointments, which were formally confirmed by the Senate, have been warmly endorsed by biomedical scientists.

Letter from Israel

from Nechemia Meyers

"ECOLOGICAL considerations are less important than the need to free Israel of dependence on oil producers for her energy supplies," a Government spokesman recently stated in reply to objections from environmentalists to plans for two new power stations, one to be fuelled by coal and the other by enriched uranium. Both represent departures for Israel, where hitherto all power plants have been fuelled by bunker oil. Naturally both pose environmental problems.

First to come into operation will be the plant at Hadera, midway between Tel Aviv and Haifa, which is equipped to burn coal and bunker oil alike. It will eventaully have four generators with an output of 1,400 megawatts, roughly equivalent to 80% of Israeli generating capacity today.

Although coal has been discovered in Sinai, its quality is evidently not very high nor is it available in sufficient quantity. More likely sources of supply are Australia, South Africa and the United States. Despite the distances of these countries from local ports, the price of imported coal, presently about $33 a ton (including transportation), would still be less than oil at $50 a ton. Officials of the Electric Corporation, which will operate these power stations as it does all others, have promised to tackle coal-related pollution problems by, among other measures, the installation of electrostatic precipitators to remove flyash.

The public is certainly more concerned about the ecological implications of the nuclear power plant than the coal fuelled one, even though, in the perhaps biased words of a physicist involved, "the latter will create pollution problems 100 times more serious than the former"

Public concern has been heightened by the recent statement of nine leading American scientists, five of them Nobel Laureates, urging President Ford to re-evaluate the US Administration's nuclear power programme in the wake of potentially serious accidents at the Decatur, Alabama nuclear power station—accidents that could occur here

Nevertheless, the 600-megawatt station will almost certainly be built, the most likely site being along the Mediterranean shore some 15 miles south of Tel Aviv. Talks are now going on with firms in the US and Europe about the purchase of a reactor, while the enriched uranium required to fuel it (if, as seems almost certain, it will run on enriched uranium) will presumably come from America in accordance with an agreement signed last year between the government of Israel and the US Atomic Energy Commission The estimated cost of the installation at today's prices is $500 million with 60% of the sum, says the Ministry of Commerce and Industry, to be spent in Israel itself on construction, equipment and labour.

● The nuclear engineers required to operate the new installation will be mainly graduates of the Haifa Technion, which has an excellent Department of Nuclear Engineering Not only has the Department trained new engineers, but is has also retrained engineers already on the staff of the Electric Corporation to handle the problems of nuclear plants

Ben-Gurion University of the Negev, located not far from the famous Dimona reactor, recently opened its own Department of Nuclear Engineering, much to the distress of the Technion, which said it could train all the engineers required at a lower cost. The Haifa school is generally unhappy about the opening of engineering faculties at other institutions of higher learning and about the decision to allow technical colleges to grant academic degrees Technion Professor Ya'acov Bar has warned that the award of academic degrees to "second-class engineers" would drastically lower the level of engineering in Israel.

● A former Technion lecturer who now holds a demanding industrial position is Dr Dahlia Greidinger, research and development Director at the Chemicals and Phosphates Company, Haifa Her staff, about half of it female, is concerned with research in two main fields: mineral feed additives to supplement the food of animals and sophisticated fertilisers. In the first category a new product, urea phosphate, is now in semicommercial production, one ton per day being turned out. Also under development is an "instant fertiliser". "In the past few years," Dr Greidinger explains, "work has been done in various countries on liquid fertilisers so that it would not be necessary to wait for rain or irrigation to bring fertiliser to the plants But liquid fertilisers are hard to ship or store So we are developing a solid fertiliser to which the farmer can add his own water when he wants to use it."

● An Israeli chemist recently teamed up with an Israeli historian to throw new light on the construction of Crusader fortifications in the Holy Land. In a report soon to appear in the *Israel Exploration Journal*, Hebrew University historian B. Z. Kedar and Weizmann Institute chemist A. Kaufman give evidence that the mortar used by the Crusaders in their large-scale construction projects contained organic materials, possibly olive oil and flax. They recall that Rufinus, a little known herbalist of the late 13th century, presented the following recipe for the preparation of mortar "Grind tiles rigourously and sift them through linsey. Then take smiths' iron-rust and grind and sift similarly, and take tow in the same quantity as of the aforesaid two, and cut it up vigorously with a lopping-knife until it will be triturated on the bench. Having done this, take equal portions of the stone and the iron-rust and put these two and the tow in a mortar, and take olive oil and mix the said cement with the said oil until it is like clay Apply it at the juncture of the stones and bind the stones together, and let it rest for many days."

They go on to quote an 18th century German missionary Stephan Schultz who, having visited Acre, reported that the extraordinary sturdiness of the Crusader palaces there stemmed from the fact that the builders used oil instead of water in preparing their mortar.

Kedar, the historian, turned to Kaufman, the chemist, to investigate the validity of this story by using carbon-14 measurements to determine whether Crusader-era mortar indeed contained organic materials Kaufman, a member of a Weizmann Institute team which provides Israel's only carbon-14 dating facility, found that 2% of the material in the mortar from Crusader buildings in seaside Caesarea was indeed of organic origin and dated from AD 1013±160 Whether or not the masons at Caesarea used the Rufinus formula, or even used flax and olive oil is far from clear.

There is no reason to believe that this type of mortar was used only in the Holy Land. French mediaeval literature, for example, contains references to mortar prepared with the blood of serpents or dragons. Unfortunately, present carbon-14 dating methods do not allow researchers to differentiate between olive oil and dragon blood.

The way you lived and died

	Australia	Canada	France	Federal German Republic	Italy	Japan	Netherlands	Sweden	UK	US
Population (millions)	13.1	22.1	52.2	62.0	54.9	108.4	13.4	8.1	56.0	210.4
Crude **birth rate** (per thousand)	18.9	15.7	16.4	10.3	16.1	19.3	14.5	13.5	13.9	14.9
GDP per capita ($s)	4,900	5,410	4,900	5,610	2,510	3,760	4,410	6,140	3,100	6,170
Death of infants in 1st year per thousand live births	16.7	16.8	12.9	20.4	25.7	11.7	11.6	9.6	17.5	17.6
Percentage of relevant age group in **higher education**	28	50	30	16	28	24	20	31	21	44
Grams of **animal protein** per inhabitant per day	71	66	68	58	45	31	56	58	56	75
Total primary **energy requirements** in tons of oil equivalent per capita	4.6	8.2	3.5	4.7	2.6	3.2	6.0	4.7	4.3	9.1
Telephones per thousand inhabitants	340	499	199	268	206	315	299	576	314	628

From 'The OECD Observer' March/April 1975.

North to Alaska

from Angela Croome

THE development of large nearshore oil deposits in the North is likely to rationalise the use of shipping in both the American and Soviet Arctic. Since the Americans have so far made no commitment to an Arctic shipping fleet and the projected Alaskan pipeline's cost has now escalated beyond all bounds there is now a good argument for reconsidering freighting under the ice in cargo submarines. Once a fleet of cargo-subs had been built the system would appear to be cheaper as well as safter than available alternatives.

The capacity to navigate the polar sea passages across North America and the Soviet Union is not properly used by either nation. The technology for bringing cargo out through the North West passage throughout the year was effectively proved in 1970 with the successful voyage of the supertanker *Manhattan*, but no use has yet been made of this solution. On the other hand, freight is regularly brought out through the Soviet "Northern Sea Route", and plans are in hand to expand the navigation despite the growth of alternative and less arduous land routes.

A North West passage to the Pacific was first attempted as long ago as the reign of Elizabeth I, and the search for it preoccupied many explorers and claimed the lives of not a few It was in the course of the search for the ill-fated Franklin expedition last century that the route was found though it was not until 1944 that a ship actually got through. The setting up and supply in the 1950s of the Canadian DEW-line (of defence radars) across northern Canada brought more traffic to the NW sea-route than ever before or since. Fifty ships and 15,000 people were being moved around the Canadian margin of the Arctic Ocean at one time but always escorted by icebreakers. It had been suggested early on that the NW passage would be more easily negotiated under the ice by submarine. An attempt was made by a First World War US submarine, the *Nautilus*, in 1931 but she got no further than half a hull's length beneath the ice. Nuclear submarines with their much longer submergence times simplified the task and in 1954 the US *Sea Dragon* negotiated the passage entirely beneath the ice. The obvious development now was for cargo-carrying submarines to bring out freight from the Canadian Arctic but there were no bulk products to bring at the time. The situation changed with the proving of oil in Prudhoe Bay, Alaska in 1968, and various alternative transport methods were considered Ice-strengthened giant tankers which could operate independently of icebreakers were an attractive possibility and in 1970 an experiment was hastily put together to test the feasibility of this idea. The largest tanker ever built in the United States, the *Manhattan*, jointly commissioned for the job at $40 million by three oil companies including BP was sliced up and the parts distributed among the shipyards of the North East US for ice-strengthening. (This was partly for speed and partly because no single shipyard could handle such a large ship 307 m long with a 45 m beam.)

Dr Charles Swithinbank now head of the Geology department of the British Antarctic Survey who accompanied the USS *Manhattan* as a member of the glaciological team on its pioneering voyage to Prudhoe Bay in 1970 recently discussed the "modern North West Passage" at a polar symposium at the National Maritime Museum, Greenwich. The object of the experiment was to establish how large a horsepower would be required to drive a 250,000-ton tanker through the ice, and so the *Manhattan* was in fact an over-powered half-scale experiment. The glaciologists made detailed measurements of the ice all along the route which was deliberately chosen to run through the toughest ice—which a normal voyage would avoid. *Manhattan*'s passage was triumphantly successful; she had no difficulty in breaking through the thickest ice and regularly preceded her icebreaker escort and a great deal of valuable glaciological and bathymetric data were collected. The conclusion was that it would be feasible for supertankers to service the Prudhoe Bay oil-wells 12 months a year.

Nevertheless analyses at the time indicated that bringing the oil out by pipeline would be cheaper, although in the event 800 miles of pipeline have been deteriorating in store at Valdez for several years, and the pipeline cost has escalated to an estimated $5,000 million. The actual cost of transporting a barrel of oil from Prudhoe by submarine cargo-ship was estimated as marginally cheaper in 1970 at 90 cents a barrel, but of course there was no such fleet available.

An almost opposite situation characterises the North East Passage—the

Soviet Union's "Northern Sea Route" in the account of Dr Terence Armstrong of the Scott Polar Research Institute, who has made a detailed study going back many years. This has been actively operated by the Soviet Union for many years and the route's administration had an almost autonomous existence until the 1930s purges. Some 400 ships ply in the area transporting 2½ million tons of freight annually. Recently a large programme of 24 specially designed medium-sized icebreakers has been laid down in Finland for the route, quite apart from the completion of the hugely powerful new atom-powered Soviet-built icebreaker *Arktika,* thought to be capable of 75,000 horsepower. In recent seasons efforts have been made to extend the duration of navigation, limited for the full 'through' route of 2½ months. A complete convoy of 13 ships actually struggled through as late as mid-January in 1972 but a policy speech by the Transport minister subsequently indicated that the experiment proved that the route could be kept open into December but not beyond (giving a September to Christmas season).

The various moves including the large new icebreaker-building programme suggests that the Northern sea route continues to have an important future despite the impression that it has been losing ground to the "southern" rail route out of Siberia where many extensions and new links have been built in recent years. The freight tonnage of the Northern sea route is holding up well, despite the increased bulk handled by the railway. Dr Armstrong points out that the sea route was developed originally for strategic reasons that never materialised. Nevertheless, having invested heavily in a system in advance of need, he concludes that the Soviet administration is loth to abandon it and expects the Northern Sea route to justify itself until the end of the century. □

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correspondence

The reward system

SIR,—A basic fallacy of your Editorial "The Reward System Needs Overhauling" of March 27 1975 is that Fellowships of the Royal Society and Nobel Prizes are not rewards but awards. They are not something a reasonable scientist can ever expect to receive as a matter of course for professional work even of the greatest merit, but a happy chance in which one can rejoice if it should happen to oneself or one's colleages.

Of course Nobel Prizes "are clearly not enough to go round". Why should they be? They are not a matter of right, a sort of career-grade for the top scientist, but an extra provided by the posthumous generosity of one man. Strictly speaking, their award is the private business of the Nobel Committee and no one else. Admittedly this Committee would be prudent to take account of public standards if confidence in the awards is to be maintained, but your criticism on this score is nullified by your own statement that Nobel Prizes "confer an unreasonable amount of credit on their recipients". Moreover it is invidious to critise the Nobel Committee for confining their awards "to a restricted number of subjects" when they have broadened their interpretation, within the terms of reference by which they are bound, to include astronomy.

It would have been best if the attack by Sir Fred Hoyle on these astronomical awards had been treated with the contempt of silence. Now that this silence has been broken, it needs to be said that whatever the occasion may have been, these Nobel Prizes are justified at least as much by decades of distinguished contribution to Radio Astronomy and Cosmology as by any single discovery. Sir Martin Ryle and Professor Hewish had created the physical facilities that made possible the original observations of pulsars, and the background of knowledge that enabled these observations to be interpreted. Mrs Burnell has a secure place in history as the Tombaugh of the discovery, and her credit has been enhanced by the calmness of the remarks she is reported to have made.

An important lesson from the whole unfortunate matter is that even very distinguished theoreticians may be profoundly ignorant of the realities of discovery in experimental science, and this calls into question the heavy reliance that has been placed on theoretical astronomers in advising SRC and other bodies on strategies for British astronomy.

It may be true that some laboratories make the "pursuit of Nobel Prizes . . . the ultimate aim". If so, the more fools they; in seeking external awards they are missing the true rewards of science. One expects scientists to have something better to do than to display the envy of socialites denied admittance to an exclusive club. If we are indeed seeing "an early shot in a battle which will set scientists against each other in years to come", it will be because they have allowed themselves to be led by journalists whose stock in trade is to whip up "controversy" As one who has neglible chance of ever becoming, let alone deserving, an FRS or a Nobel Laureate, I shall continue to live without envy of those on whom this fortune falls, and I think well enough of my colleagues to believe that the majority of them will do the same.

P. B. FELLGETT
University of Reading, UK

A hundred years ago

THE Rev. G. H. Hopkins gives the following method for fixing the curves which steel filings take when under the action of a bar magnet. The filings having been prepared so as to be as nearly the same size as possible, and that size very minute, are pound into a mortar, and a small quantity of finely powdered resin is added; these are stirred together until the two substances are completely mixed, and then, considerable pressure being exerted upon the pestle, they are rubbed until the resin adheres to the filings in a very fine coating. The filings can then be sprinkled as usual, and the curves formed. It is best (after the curves are formed) to heat the plane surface, glass, paper, or wood, according to convenience, over a stove or in an oven, which easily allow it to be sufficiently as well as uniformly heated. For projecting the curves on a screen, the following, we believe, is a very effective method. Cover the glass with thin gum-water, allow it to dry perfectly; obtain the curves on the dry gummed surface; finally, breathe on the plate: the gum is thereby softened and the curve permanently fixed. Substituting corresponding shaped pieces of paper for the magnets (a pin-hole can be used to indicate the N. pole), the curves can be covered with a second plate of glass, and thus preserved as an ordinary lantern slide.

from *Nature*, **12,** 97; 3 June, 1875

news and views

SV40 *A* gene required to maintain transformation?

from a Correspondent

CELLS transformed by certain temperature sensitive (ts) mutants of Rous Sarcoma Virus (RSV) are temperature dependent in their transformed phenotype (Martin, *Nature*, **227**, 1021; 1970; Wyke and Linial, *Virology*, **53**, 152; 1973). Thus RSV viral genes are necessary for the maintenance of the transformed state. The *A* gene of SV40 is known to be required for viral DNA synthesis during the lytic cycle (Tegtmeyer, *J. Virol.*, **10**, 591; 1972; Kimura and Dulbecco, *Virology*, **52**, 529; 1973; Chou *et al.*, *J. Virol.*, **14**, 116; 1974). The *A* gene function is also needed for the initiation of transformation (Tegtmeyer, *ibid.*; Kimura and Dulbecco, *ibid.*) but was thought not to be required for the maintenance of the transformed state (Kimura and Dulbecco, *ibid.*). In the last few months a number of papers have appeared which strongly suggest that cells transformed by ts mutants in the *A* gene are ts for at least some of the phenotypic markers of transformation (Kimura and Itagaki, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 673; 1975; Martin and Chou, *J. Virol.*, **15**, 599; 1975; Tegtmeyer, *J. Virol.*, **15**, 613; 1975; Brugge and Butel, *J. Virol.*, **15**, 619; 1975; Osborn and Weber, *J. Virol.*, **15**, 636; 1975). All these papers confirm that *A* mutants are temperature sensitive for the initiation of transformation.

The cells studied include primary rat (Osborn and Weber), a continuous rat line (Kimura and Itagaki), the continuous mouse cell lines Swiss 3T3 (Tegtmeyer) and BALB 3T3 (Brugge and Butel), Syrian hamster embryo (Tegtmeyer, Brugge and Butel), Syrian hamster tumour cells (Brugge and Butel), Chinese hamster lung cells from a single animal (Martin and Chou), rabbit kidney (Tegtmeyer and Butel), and human cells from a patient with Franconi anaemia (Brugge and Butel). The Chinese hamster lung, rabbit kidney and human cells are semipermissive for SV40 whereas all the other cells are nonpermissive. The *A* mutants used were either isolated by Tegtmeyer (*J. Virol.*, **10**, 591; 1972) (Tegtmeyer, Brugge and Butel; Osborn and Weber), by Kimura and Dulbecco (*Virology*, **52**, 529; 1973) (Kimura and Itagaki) or by Chou and Martin (*J. Virol.*, **13**, 1101, 1974) (Martin and Chou).

In general, cells are transformed at the permissive temperature (usually 33 °C, but 37 °C is used by Brugge and Butel for hamster embryo and human cells) by an *A* mutant and its corresponding wild type. The transformed cells are then tested for their transformed nature at the permissive and nonpermissive (39–41.5 °C) temperatures. The behaviour of the *A* mutant transformed cells are compared to those of wild type transformed (in some cases also cells transformed by late ts mutants) and untransformed cells at these temperatures.

Unfortunately this experimental procedure is not always used. Kimura and Itagaki use cells which have been transformed by wild type virus at 37 °C and which are cultivated at 37 °C until just before being tested. Brugge and Butel use a line of BALB/c embryo cells which were transformed at 37 °C by a different wild type strain (not that from which the *A* mutants were isolated) for comparison with their *A* mutant transformed BALB 3T3 cells. They also use a Syrian hamster tumour line which has also been transformed by a different wild type strain and carried for a long time *in vitro* and *in vivo* to compare with their *A* mutant hamster tumour lines.

Individual transformed clones isolated at the permissive temperature (except for the wild type clones of Kimura and Itagaki) are used by Martin and Chou; Osborn and Weber, Kimura and Itagaki. Both Tegtmeyer and Brugge and Butel used uncloned populations of cells which after infection with virus and subculture at the permissive temperature are deemed to be transformed by their morphology and the presence of T antigen. Thus these two groups are looking at mixed populations of transformed cells.

Criteria for transformation

In general transformed cells are not contract inhibited and grow in a disorganised manner forming dense multilayers and reaching high saturation densities Normal cells are contact inhibited and grow in an oriented manner usually forming only a monolayer of cells. Transformed cells (especially those not derived from continuous cell lines) tend to have higher efficiencies of plating (ability to form colonies) than normal cells under a variety of conditions.

The exact definition of a transformed cell has always plagued papovavirus workers and a variety of criteria have been used by the different authors. These include non-contact inhibited morphology (Brugge and Butel, Tegtmeyer, Osborn and Weber), saturation density (Osborn and Weber, Brugge and Butel), colony formation at low cell density in high serum (Tegtmeyer, Brugge and Butel), in low serum (Martin and Chou), in agar (Kimura and Itagaki, Brugge and Butel) and on top of a monolayer of untransformed cells (Brugge and Butel; Martin and Chou), growth rate in low serum (Kimura and Itagaki), presence of T antigen (Osborn and Weber; Tegtmeyer, Brugge and Butel) and increased uptake of hexose (Brugge and Butel).

Using six different *A* mutants Martin and Chou found eight out of nine of their *A* mutant clonal lines are ts for colony formation at low cell density in low serum and for their ability to form morphologically distinct colonies on top of untransformed monolayers. Unfortunately these are the only two tests used and one wonders what happens to colony formation in high serum and if morphological changes do occur at the high temperature.

Kimura and Itagaki find that three independently transformed clones, transformed by one *A* mutant, are ts for colony formation in agar and on top of untransformed cell monolayers. They also report that three clones grow less well in low serum (but not in high serum) at the nonpermissive temperature, but this result is not terribly convincing. Also as stated above they do not use adequate wild-type transformed cells for comparison.

Osborn and Weber present data for only one *A* mutant transformed clone and one wild type transformed clone derived from primary rat kidney cells They observe that the *A* mutant clone is ts for morphology, alignment of actin fibres, saturation density and the presence of T antigen. Osborn and Weber find that the number of *A* mutant transformed cells containing T antigen decreases (15% positive) after 3 days at nonpermissive temperature. Tegtmeyer and Brugge and Butel do not see any decrease in *A* mutant transformed cells containing T antigen at the nonpermissive temperature, but it is not clear how long they kept their cells at high temperature before testing for T antigen. It is not obvious that Osborn and Weber can fruitfully compare their two clones since the cells they used were derived from heterogenous rat primary cells and different cell types may have been transformed by the different viruses. Many more transformed cells must be isolated and tested before much meaning can be given to these results.

Brugge and Butel study a variety of parameters of their *A* mutant-transformed lines including morphology, saturation density, cloning in agar and on top of untransformed monolayers, the uptake of hexose and the presence of T antigen. They find that all their *A* mutant lines tested are ts for these parameters except for one human line which is not ts for the increased uptake of hexose and that all the lines retain T antigen at the high temperature. Unfortunately not every *A* mutant line is used for every test and as mentioned above some of the wild type transformed cells used are not adequate for comparison with the mutant lines either being of the wrong cell type, being transformed and maintained at the wrong temperature or being transformed with the wrong wild type strain. It is interesting to note that *A* mutant tumour lines which were formed *in vivo* at 37 °C are found to be ts at 39.5 °C.

Tegtmeyer's results are somewhat in disagreement with those of Brugge and Butel although the same *A* mutants and in some cases in the same cell types and parameter of transformation are used. He finds that none of his *A* mutant transformed mouse lines are ts for colony formation in high serum. Only rabbit kidney cells transformed by one mutant (A28) are ts for colony formation in high serum (rabbit kidney cells transformed by five other *A* mutants are not ts). The temperature of 41.5 °C, used by Tegtmeyer for his rabbit kidney cell experiments, is the highest nonpermissive temperature used by any of these workers. Tegtmeyer does find that hamster cell lines transformed by five out of six *A* mutants are ts for colony formation at low cell density, but not at high cell density. All Tegtmeyer's *A* mutant transformed lines retain T antigen at the high temperature. He does note that all *A* mutant transformed cells of either mouse, rabbit or hamster that grow at high temperature retain their transformed morphology. Tegtmeyer concludes that the temperature sensitivity of the different *A* mutant transformed phenotypes may well depend somewhat on the virus–cell interaction which may vary. Both Tegtmeyer and Brugge and Butel use uncloned mixed populations of transformed cells which contain cells derived from more than one transformation event. It is not clear whether different populations are showing up in the different parameters measured and whether different results would have been obtained if clonal lines had been tested.

Even though there may be some reservations about some of the results presented, and no one paper is entirely convincing, in general all these papers taken together seem to indicate that the *A* gene of SV40 is responsible for at least some of the phenotypic markers of transformation. It certainly seems clear that the induction of transformation by *A* mutants is much more ts than the possible maintenance of the transformed state. Those *A* mutant lines which are not very ts may have been transformed by a wild type revertant, or a less ts variant of the mutant. Alternatively, these lines may possibly contain a spontaneous transformation event (which is not ts) superimposed on the viral transformation or may contain many mutant genomes which together can overcome the ts phenotype. It would be interesting to test the ts phenotype of cells transformed by virus rescued from such lines. It is clear that a lot more careful work will have to be performed before a maintenance function can definitely be assigned to the SV40 *A* gene.

Early history of agriculture

from Don Brothwell

It is something of an event when the Royal Society and British Academy get together to consider topics of mutual interest. The history, more correctly prehistory, of agriculture in relation to developing earlier cultures is such a field of interest. As a veteran symposium attender, perhaps I should have known that the symposium held at the Royal Society on April 9 and 10 on early man as a farmer would be a disappointment. But some papers were more useful contributions than others; responses were also varied, some being more favourable than my own. The organisers gathered together contributors to cover a wide range of topics, and if the speaker weighting was too heavily towards Cambridge it was at least an example of sample bias which can occur in mammal assemblages related to archaeology.

Following introductory remarks by J. G. D. Clark, J. R. Harlan (University of Illinois) usefully considered the distributions of wild progenitors of domestic animals and plants, as a possible basal reference point from which to consider the ecological adaptions of domesticated forms. The picture is a complex one, in which it is necessary to appreciate such variables as possible climatic change, the varying influence of human societies, and the extent of further microevolution which might have occurred even in the wild stocks. Harlan viewed patterns of variation among species, attempting a classification into so-called endemic, monocentric, oligocentric and noncentric forms. He emphasised that for a number of domesticates, there had been multiple centres of evolution—a fact as yet not fully appreciated.

W van Zeist (State University of Groningen), who followed, paid special attention to the early food plants of south-western Asia. The earliest crop plants in this area prior to 6000 BC included barley, wheat, pea and lentil. By 5000 BC the oil plant linseed was also in cultivation. Palaeoethnobotany has its own range of problems, and the nature of ancient plant material presents considerable difficulties in identification of the species. Van Zeist also pointed out that although pollen studies have been extremely valuable in the reconstruction of ancient environments in this part of Asia, its direct contribution to the study of earlier agriculture is likely to be far more limited.

Some indication of the significance of studying material culture in relation to early domestication and type of economy was given by A. Steensberg (University of Copenhagen) Although an essential part of any reconstruction of early agriculture, he nevertheless pointed out that there were various problems of interpretation—the 'polish' on flint sickles doesn't necessarily indicate cereal cultivation, and so-called paddle-shovels could have had varied uses. Spades and spade marks, he considered, were the first clear proof of husbandry. He reasonably argued that an idea could have remained latent in a community until other conditions were right for its successful application. Thus the antiquity of a positive cultural innovation may not be clearly related to the prehistory of the idea and rationale. Rather surprisingly, Steensberg was the only one to consider population pressure as a possible determinant of cultural change, and in fact concluded—wrongly I believe—that it has not been a significant factor.

Clearly it was important to have the genetic aspects of crop plant evolution considered in such a symposium, and this was done by Barbara Pickersgill (University of Reading) and C. B. Heiser. They pointed out that domestication led to greater morphological variation than is seen in wild progenitors, but that unique processes were not involved. Moreover, the influence of man as a selection pressure may have changed through time—a fact to be revealed if at all by the archaeological investigation of earlier human communities and their implements. Although cytogenetic aspects of plant evolution have been explored to some extent in relation to wild plants and cultivars, relevant physiological adaptions are by no means worked out L. T. Evans (CSIRO, Canberra) outlined some of the data and problems in this field—in so far as they might be relevant to studies on ancient cultivars. He indicated that there have been changes in life cycles, degree of environmental control and yield potential in some plants—related to factors of climate, basic anatomy, and hormone variation—but the physiological basis of such changes was not well understood. Human intervention does not seem to have produced an increase in relative growth rate in the various plant species.

In contrast to the previous papers which were mainly concerned with plants, the next two were a slightly limp attempt to restore balance and give more consideration to animal populations.

M. R. Jarman (University of Cambridge) gave general comment on the development of early animal husbandry He attempted to show the differences between zoo-archaeology and archaeo-zoology, one being concerned with earlier human behaviour in relation to other animal species, while the zoological approach was perhaps more strictly to view the nature of microevolutionary change from wild to domestic forms within a particular species. He emphasised that we must settle into appreciating that animal husbandry in its most general sense occurred well before the Neolithic culture phase. Moreover, it is important to examine the possibility of close man–animal relationships in prehistory beyond the scale of domestic relationships seen today

As yet, biochemical taxonomy has had only modest recognition in helping to resolve questions of domestication There is no doubt that it has far greater potential, and it was good that at least honourable mention was given to this field at the conference. E M. Jope (Queens University of Belfast), in fact, concentrated on animal proteins, and spent much of his allotted time in introducing this complex field. He did however indicate clearly that various lines of investigation may eventually produce significant results, for instance, the haemoglobins and other biochemical variation in modern animal stocks, amino-acid differences in ancient bone, and perhaps even ancient and recent keratins.

Except for the concluding paper, the rest of the contributions were concerned with regions, and in some cases with particular crops. G. H. S. Bushnell (University of Cambridge) outlined the beginnings of agriculture in the Mexican area. Although he is the doyen of Americanists in this country, it was a little regrettable that those who had more direct acquaintance with the significant developments in the agricultural history of the New World were not in attendance at the meeting. Bushnell did make the pertinent point that the long, and probably ecologically complex, development of agriculture in the Americas could hardly be seen as a "revolution" in Childe's sense of the term.

Sir Joseph Hutchinson's task was to consider crop plant evolution in the Indian sub-continent. He indicated that the various plants of economic value to the earlier cultures had a diverse origin—being introduced at various times from Asia to the east and from both south-west Asia and Africa to the west. Tea was one of the few crop plants which was probably indigenous. Possible trans-Pacific movement of plants was considered in relation to maize. In contrast to these plant origins, Hutchinson believes that livestock may not have been so derived from elsewhere—wild forms of domestic varieties being still seen in India. Early agriculture in this area, Hutchinson suggests, is best seen as an extension of that evolved in Western Asia, and certainly there were links into Mesopotamia. Finally, Hutchinson pointed to the considerable variation which had occurred in some plants, such as the banana, cotton, wheats and rice—not so much an indication of the degree of domestication as the nature of the breeding system, length of time the crop was under cultivation, the cumulative nature of certain mutations, and even the response to disease. Further aspects of early Asian agriculture, and particularly rice cultivation, were explored by T T Chang (International Rice Research Institute, Philippines). As he said, rice now feeds more people than any other cereal and clearly has an intimate relationship with the development of some cultures, especially in eastern Asia. Varieties of rice appear at different dates. In some rice cultures, millet was in fact the oldest crop; an

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Evidence for triggering

from Peter J. Smith

As long as the nature of deep earthquakes remains unclear, wisdom would seem to dictate emphasis on the study of single-source events. Yet multiple events, though more complex, give additional data which can be put to good use Both Chandra (*Bull. seismol. Soc. Am.*, **60**, 639; 1970) and Fukao (*Phys. Earth planet. Interiors*, **5**, 61; 1972), for example, were able to use multiple-source earthquakes in the Peru–Brazil region to determine rupture velocities. In each case the secondary events apparently lay on a nodal plane of the primary shock, from which it was assumed that the fault plane was continuous between epicentres and that rupture was planar and unidirectional.

Clearly, however, such analyses beg the question of earthquake mechanism—a problem given point when Dziewonski and Gilbert (*Nature*, **247**, 185; 1974) showed that for some deep shocks the double-couple mechanism is not entirely adequate. Moreover, although Chandra and Fukao obtained relative locations and origin times of the secondary events, they did not determine the fault plane solutions. This omission could be critical, for Strelitz (*Geophys Res. Lett.*, **2**, 124; 1975) now shows that it may have led to false assumptions.

Using both P and pP phases, Strelitz has analysed the 5 September 1970 Sea of Okhotsk earthquake which comprised a magnitude 4.5 event followed about 5 s later by a magnitude 5.7 event with roughly the same epicentre but a focal depth 23 km greater. He finds that the fault planes and principal stress axes of the two events were significantly different and that the second event lay on neither of the nodal planes of the first. A curved rupture zone seems to be ruled out on the grounds that such faulting would probably radiate continuously during rupture, whereas the two shocks observed were discrete events. That the proximity of the two events is purely coincidence also seems unlikely because other similar event pairs occur in the same region. Strelitz thus concludes that the first shock simply triggered the second.

alternative being mixed cropping as an insurance against climate failure. Intimately bound up with the success were the emerging techniques of irrigation, terracing, fallowing, weed control and manuring. A major question in resolving the development of rice cultivation is concerned with the contrasting styles of upland and lowland rice cultures, although their relative antiquities, Chang feels, cannot be resolved at present.

The contributions given by E. S. Higgs and Heather Jarman (University of Cambridge) were both concerned with the development of prehistoric agriculture in Europe, either in the uplands or lowlands (where resources tend to differ from one another). Both were involved with the construction of a theoretical framework concerned with possible changes in relations between populations, natural resources and the level of technology. Patterns of agricultural exploitation, they consider, can be demonstrated from divergent regions of Europe. This territory approach is one of the methods particularly adopted by the Cambridge group, and deserves fully testing on actual site data. Unfortunately theoretical frameworks, although worth exploring, are always in danger of showing the 'average' rather than the highly variable nature of human society.

As a classical archaeologist, J. Boardman (University of Oxford) provided something of a contrast to the majority of other papers. He demonstrated that this zone of archaeology can contribute usefully to a full reconstruction of plant use in relation to earlier human cultures. Although the olive was dealt with in particular detail, various plants and animals could have been similarly treated. Wild olive grew freely in the early Mediterranean, and its value was well recognised by Bronze Age times—when it became important economically in Greece at least. The elaboration of a material culture to cope with the extraction and uses of olives even included special scrapers for the removal of oil after its application to the body!

The concluding paper of the conference provided a worthwhile contrast, more precisely an alternative viewing of the relationship between man and his environment during these earlier cultural phases. G. W. Dimbleby (University of London) pointed out that although during his evolution man had 'freed' himself to some extent from his natural environment, he has probably influenced it profoundly in some areas—although to a varying degree depending on the nature of the human activity within the ecosystem. In particular agriculture, both pastoral and arable, has been a significant imposed change on the ecosystem—with resultant changes to the components of the microclimate and vegetation. Eventually, even soil degradation and marked erosion can occur, and then it is increasingly difficult for regeneration processes to occur. Holocene environments, then, are by no means in a state of equilibrium, and some are far more 'brittle' than others. It would also appear that often the early farmers were in fact concentrating on these brittle environments, such as savanna woodland, steppe, or light deciduous forest—and there is growing evidence of the detrimental side effects which eventually occurred.

X-ray diffraction studies of biomembranes

from A. E. Blaurock and W. R. Lieb

MEMBRANE biochemists, following the maxim that "the proper study of mankind is man", have studied the human red blood cell membrane in great detail. Unlike other human tissues, blood can be obtained in large quantities without harming the donor. There is the added attraction that the red cells have no internal membranes to complicate matters And pure cell membranes are easily obtained by haemolysis, that is by osmotically bursting the cells and extruding their contents into hypotonic solutions. The resulting 'ghosts' are single-walled sacs of cell membranes.

It has been found that most of the ghost protein will react with aqueous labelling reagents and enzymes when these are allowed to enter the sac, but little reacts when these are kept outside. The inference which membrane biochemists have drawn from such results is that most of the protein is associated with the inner, cytoplasmic surface of the membrane (for a recent review, see Steck, *J. Cell Biol.*, **62**, 1–19; 1974). In addition, about a third of the total protein mass is released at very low ionic strength without detergents, suggesting a location largely extrinsic to the lipid bilayer Interestingly, this superficial protein (mainly 'spectrin') reacts exclusively with inside reagents. The picture that emerges is of a very asymmetric membrane, with more protein on the inside than on the outside and with much of this inner protein lying superficially at the cytoplasmic surface. With this picture in mind, it is surprising that a recent X-ray diffraction study by Stamatoff, Krimm and Harvie (*Proc. natn Acad. Sci U.S.A.*, **72**, 531–534; 1975) concludes that the electron density profile for the ghost membrane is symmetrical.

Although ghost sacs in unoriented dispersions have been looked at by X-ray diffraction, Wilkins *et al.* (*Nature new Biol.*, **230**, 72–76; 1971) also pointed out the advantages that would come from arranging the membranes in

A new anti-sickling agent?

from a Correspondent

CURRENT interest in the fundamental aspects of the aggregation of deoxy sickle-cell haemoglobin is but one approach to the serious practical problems which arise in the management of sickle cell anaemia patients. Side by side with studies on the behaviour of deoxy Hb-S there have been attempts to devise therapeutic methods for reducing or abolishing the sickling process *in vivo*. Proposals to use urea and, more recently, cyanate as anti-sickling agents have been widely publicised and their effects on the aggregation of deoxy Hb-S are susceptible to rational explanation. The value of cyanate as a long-term anti-sickling agent is now being very carefully assessed by US workers. Less has been heard recently of the anti-sickling properties of steroids, although both testosterone and progesterone have been tested *in vitro* and *in vivo* and claimed to have some degree of efficiency.

Now comes the news of an entirely novel type of anti-sickling agent, found in aqueous extracts of *Fagara zanthoxyloides* root. In their first publication in a European journal, a group of workers in Ile-Ife and Ibadan, Nigeria, report some toxicological and preliminary clinical trials of this material (Isaacs-Sodeye, Sofowara, Williams, Marquis, Adenkunie and Anderson, *Acta haemat.*, **53**, 158; 1975). The roots in question are used as chewing sticks in the Western State of Nigeria and then the fibrous end thus produced is used to brush the teeth. There is evidence of antimicrobial activity against oral microorganisms in the root extract and no folk history of side effects.

The paper intimates favourable toxicological findings in limited tests on *F. zanthoxyloides* extracts, and a significant diminution in the incidence of painful episodes (sickle-cell crises) in the few patients treated Comprehensive clinical trials at many centres in Nigeria are now being organised. The results of these trials will be awaited with great interest, especially as it is stated that a pure component of the root extract with potent anti-sickling activity can also be synthesised in the laboratory.

regular, oriented stacks. Even if the membrane is asymmetric, the collapsed sac will then form a symmetrical structure, greatly simplifying the determination of the membrane electron density profile. In addition, in-plane diffraction is then seen separately from profile diffraction. These considerations were also in the minds of Stamatoff *et al.*

Their ghost membrane sacs were prepared in the usual manner. To orient the membranes, the sacs were then spun for a long time at a very high speed (24 h at 100,000 *g*, 4 °C), and excess water was removed by equilibrating in a nitrogen atmosphere at high relative humidities. The spacing between membranes was varied by a final equilibration at 75–100% relative humidity. The presence of sharp diffraction arcs showed that these preparations indeed contained oriented membranes in regular stacks. The repeat distance was 56 Å at the lowest hydration, increasing to 70 Å when more water was present. If, as the biochemists argue, the membrane profile is asymmetric, one expects the repeat distance to include two membranes, since the collapsed sac is then the symmetrical unit. But current ideas of biological membranes are based upon a bilayer arrangement of the lipids, and a bilayer thickness of less than 28 Å is unlikely Therefore the authors reasonably conclude that there is only one membrane in the repeat unit, with a thickness of some 56 Å. Assuming that the ghost sacs are intact, they further conclude that the erythrocyte membrane has a symmetrical electron density profile.

At present, then, there seems to be a contradiction between this interpretation and the biochemical findings, and one must look for ways of reconciling the experimental results. The authors point out that, in general, a membrane which is biochemically asymmetric may nonetheless have a symmetrical electron density profile. But the weight of biochemical evidence points to there being much more protein on the cytoplasmic than on the extracellular side of the ghost membrane. It may be that the biochemical evidence has been misinterpreted. But it is more likely, in our view, that the native structure of the ghost sacs was altered during the fairly vigorous steps required to orient the membranes. Knutton *et al.* (*J. Cell Sci.*, **7**, 357–371; 1970), in a similar X-ray study, did report such alterations, although the procedures were not identical to those of Stamatoff *et al.* Support for this possibility also comes from the latter group's calculated electron density profiles. They are rather similar to those published for egg lecithin/cholesterol bilayers (Levine and Wilkins, *Nature new Biol.*, **230**, 69–72; 1971). The peaks at the presumed lipid headgroup regions are narrow. In addition, when much of the water is removed from between the membranes, peaks in neighbouring profiles come right up to each other. Thus there is little indication of the large amount of superficial protein, such as the 'spectrin', present in the native membrane.

Clearly, for the future, it would be very valuable to study possible changes in membrane structure during each stage of the preparation of oriented specimens. For example, electron microscopy could be used to check for fragmentation of the ghost sacs. Since most biological membranes are not naturally oriented, a successful resolution of these difficulties would greatly extend the application of X-ray diffraction techniques.

How do animal cells communicate?

from John D. Pitts

An International Titisee Conference on "Information Transfer between Cells", sponsored by the company of Karl Thomae and chaired by W. R. Loewenstein (University of Miami), was held on April 24–27.

DIRECT communication between animal cells through junctional channels which join the cytoplasms of adjacent cells has now become accepted as one of the important and fundamental mechanisms of intercellular communication. Communication is necessary for the coordination and control of growth, pattern and function in multicellular systems and the different forms of communication, including surface–surface interactions, communication by extracellular signalling molecules and junctional communication, may all play a part.

Small molecules and ions move freely through intercellular junctions which means that the cell has lost its status as the independent unit of animal tissues, though its individuality is maintained by its macromolecules which do not pass through junctions and are therefore restricted to the cell in which they are made. At this interesting time, when ideas and attitudes are being modified, the Titisee conference sorted out some of the ground rules and established from anatomical, physiological and biochemical approaches what are the generally accepted properties of junctional communication.

Junctional permeability can be detected either electrophysiologically or biochemically and the site of permeability has been correlated with the morphologically identifiable gap junction These junctions appear as arrays of closely packed (hexagonally packed in fixed preparation) subunits, 80–90 Å in diameter which traverse the two closely apposed membranes. N. B. Gilula (Rockefeller University, New York) and D. A. Goodenough (Harvard Medical School) described the structure of the junctional subunit as a dumb-bell-shaped protein complex penetrated along its length by an aqueous channel 15 to 20 Å in diameter. The junctional proteins are not glycosylated and can be separated into two components of molecular weight 25,000 and 15,000.

The permeability characteristics of these junctions are consistent with the idea of non-specific transfer through simple aqueous channels. Electrophysiological measurements show free movement of ions between cells joined by gap junctions (hence the term low-resistance junctions). Injected dyes such as flourescein diffuse through junctions and J. D. Pitts (University of Glasgow) reported that nucleotides, sugar phosphates, choline phosphate and the cofactor tetrahydrofolate are exchanged freely between junction-forming cells in cuture. So the picture emerges that many (perhaps all) small cellular ions and molecules pass through junctions. But there is a size limit on transfer: junctions are not permeable to proteins or nucleic acids, although in contradiction to Pitts, G. M. Kolodny (Harvard Medical School) claimed that RNA is transferred between mouse 3T3 cells, but only in very confluent culture.

Evidence for modulation of junctional permeability was presented by B. Rose (University of Miami) and W. R. Loewenstein. They have correlated a reversible increase in junctional electrical resistance with transient increases in intracellular calcium concentration. The idea of controlled permeability is attractive although as yet there is no evidence for such control occurring under physiological conditions.

Intercellular growth control mediated by junctional exchange of metabolites has been observed in simple culture systems but data by M. G. P. Stoker (Imperial Cancer Research Fund, London) suggest that not all forms of growth control depend on junction formation. The loss of topoinhibition at wound edges in culture is apparently not due to loss of junctional communication, so it seems, perhaps not surprisingly, that more than one form of communication is involved in cell growth control.

Another important form of direct communication between cells is mediated by surface–surface interactions which may well involve carbohydrate groupings Y. C. Lee (Johns Hopkins University, Baltimore) and H. Wiegant (Philipps University, Marburg) reviewed the structure and properties of membrane glycoproteins and glycolipids and R. C. Hughes (NIMR, Mill Hill, London) described cell variants with specific glycosylation defects. Such cells will provide valuable tools for studying the functions of cell surface carbohydrates. M M. Burger (University of Basel) described a proteoglycan-type aggregation factor and a second factor (termed baseplate) isolated from sponge cells. If baseplate is covalently linked to sepharose beads, the beads aggregate in the presence of aggregation factor. Further analysis of this simple system could lead to an understanding of the molecular mechanisms involved.

The role of cell movement and cell contact in embryogenesis was emphasised by A. Moscona (University of Chicago) who suggested that re-aggregation of dissociated cells provides a useful model system for the study of this process. He has isolated tissue-specific glycoprotein ligands (cognins) which mediate specific cell reaggregation in the chick neural retina system.

Communication mediated by extracellular factors may also play an important part in growth control and differentiation. W. J. Rutter (University of California, San Francisco) described the effect of a factor isolated from mesenchyme which can regulate the differentiation of pleiotropic precursor cells in primitive rat pancreas. The factor seems to act at the cell membrane and has two functional components. One, which is periodate sensitive, acts by increasing internal cyclic AMP and the other has a separate but unknown function. M. C. Raff (University College, London) pointed out that there are two classes of extracellular signalling ligand, those which act monovalently altering the activity of a membrane receptor and those which act multivalently causing receptor clustering. In two cases of signal transduction by receptor clustering—mast cells treated with specific antigen or anti-IgE and T lymphocytes treated with certain mitogens—there is a rapid (in less than a minute) and transient increase in calcium uptake (see page 378 of this issue). Raff suggests that ligand binding opens membrane calcium channels and that increased cellular calcium levels initiate (in some unknown way) the further events leading to histamine release or DNA synthesis

In the slime mould G. Gerisch (Friedrich-Miescher Laboratory, Tübingen) has shown that extracellular cyclic AMP pulses stimulate the cellular cyclic AMP generating system by a feedback mechanism and thus the necessary amplification is provided to relay cyclic AMP-mediated signal from one cell to the next This type of mechanism for maintaining signal level along a chain of cells could clearly be of more general importance.

The specificity of synapse formation must depend on some form of intercellular communication, as yet unknown. A number of people have approached this central problem of neurobiology from different angles. J. G. Nicholls (University of Stanford) has defined the detailed cellular organisation of leech ganglia and is studying synapse regeneration in organ culture. M. Nirenberg (National Institutes of Health) has isolated and partly characterised a number of neuroblastoma cell lines with defective neural gene products. J. P. Changeux (Institut Pasteur, Paris) has made a detailed study of the cholinergic receptor protein. C. Levinthal (Columbia University) has described, in three dimensions, the growth of particular nerve fibres towards specific target neurones or neuroblasts. M. Jacobson (University of Miami) has studied the temporal sequence of events leading to the establishment of specific retinal tectal connections in amphibia. S Ward (Caltech) has isolated behavioural mutants of a nematode which fail to respond to a variety of chemical attractants. Anatomical changes have been identified in the terminals of the sensory neurones of mutant worms in four out of six complementation groups Mutants in three of these groups are sterile so if sterility and the sensory change result from the same molecular defect, then the biochemical analysis of sperm (which are easily isolated) may lead to an understanding of the neurological defect.

The concept of successive subcompartmentation during insect development was reviewed by P. Lawrence (MRC, Cambridge). The division of one compartment into two subcompartments appears to be associated with the expression of a single gene in one subcompartment The *engrailed* gene, expressed by cells in the posterior wing compartment of *Drosophila* distinguishes these cells from those in the anterior compartment Clones of rapidly growing cells with a defective *engrailed* gene (produced by genetic tricks) which appear in the anterior compartment respect the anterior–posterior compartment boundary, whereas clones appearing in the posterior compartment do not.

The formation of gradients of positional information, which specify pattern during development, also depends on intercellular communication. If it depended on the movement of signal molecules through intercellular junctions, it might perhaps be expected that restricted permeability should be found at the compartment boundaries Lawrence has examined the insect segmental boundary and finds no electrophysiological or morphological evidence for discontinuity of junctional coupling.

A. Gierer (Tübingen) proposed that gradients of chemical factors, rather than intrinsic cell polarity, determine the polarity of regeneration in *Hydra*. L. Wolpert (Middlesex Hospital Medical School, London) suggested that in the developing chick limb position along the anterio–posterior axis is probably specified by a gradient of information originating from a region near the posterior margin, but that along the proximo–distal axis position is specified by the time cells spend (number of divisions made) in a progress zone at the growing tip of the limb bud.

Intercellular communication is clearly important in multicellular organisation, but which form of communication has which role is yet to be determined.

Interfering with cancer

from Mathilde Krim, Arthur S. Levine, Thomas C Merigan and Jan Vilcek

An international workshop on interferon in the treatment of cancer, held in New York on March 31–April 2, was sponsored by the Memorial Sloan–Kettering Cancer Center and the Division of Cancer Treatment of the National Cancer Institute. The meeting was attended by workers from a number of European countries and by many American workers, including representatives from the Institute of Allergy and Infectious Diseases, whose anti-viral programme has sustained work in the USA for several years.

INTERFERON, a naturally produced anti-viral protein, inhibits the replicative and transforming activity of both DNA- and RNA-containing tumour viruses But its *in vivo* effect on tumours apparently cannot be explained solely by its anti-viral action. Ion Gresser (Institut de Recherches Scientifiques sur le Cancer, Villejuif) and others reported that mouse interferon delays the appearance or retards the spread of mouse virus-induced leukaemias and certain solid tumours, in some of which there is no demonstrable viral involvement.

One investigator reporting at this meeting, however, Richard Adamson of the National Cancer Institute, stated that he had been unable to demonstrate an anti-tumour effect in his L1210 mouse leukaemia system. But interferon has been shown to be capable of protecting mice in instances of tumour induction by radiation and by the chemical carcinogen methylcholanthrene. Should interferon have anti-tumour effects *in vivo* operating through mechanisms other than those concerned with inhibition of viral proliferation, experimental evidence has suggested that these could be: inhibition of tumour cell division, changes in tumour cell surface properties, modulation of lymphocyte functions, enhancement of macrophage phagocytic activity and changes in serum complement level. The relative importance of these effects is still unknown; in some cases interferon might act directly on the tumour cells; in others it seems to modify components of the immune system.

Martin Hirsch of the Massachusetts General Hospital in Boston reported that in chimeric mice (models for human organ and bone marrow transplantation) mouse interferon reduces C-type virus production, lymphoma incidence and the severity of graft-*versus*-host reactivity. He and Edward De Maeyer, Institut du Radium, Orsay have also shown that it delays skin graft rejection.

In the 1960s, a number of clinical trials with synthetic interferon inducers administered to patients were inconclusive in that inducers were found to have both toxicity and limited effectiveness with regard to the level of interferon they elicited in man. The alternative, the large-scale production of exogenous human interferon by cultured cells, was pursued in a number of laboratories, particularly at the Central Public Health Laboratory in Helsinki. Here Kari Cantell has used leukocytes obtained from blood bank buffy coats as interferon producers, and has perfected a system of obtaining high titre interferon preparations suitable for clinical use. Now, with Hans Strander of the Karolinska Institute, he has begun to explore the use of his product in the treatment of a variety of human cancers.

Late in 1974 Strander reported that in a small group of patients with osteogenic sarcoma treated over the past two years surgical removal of the primary tumour, followed by long-term administration of interferon, seemed to have warded off the occurrence of metastases (National Cancer Institute Monograph, in the press). The high titre exogenous interferon he uses seems to be devoid of overt toxicity.

These early results provided the impetus for the workshop, which was designed to review them critically. A careful review by Dr Strander of his clinical evidence was followed by the report by Alan Rabson (National Cancer Institute) on two independent reviews of the pathology of the treated Scandinavian cases. The originally malignant nature of all the tumours was confirmed, but it was felt that statistical analysis could be based only on that bone tumour type for which solid prognostic data exist, agreed by all to be classical osteogenic sarcoma. It was concluded that the rate of recurrence of disease in the form of lung metastases in the cases so selected did appear reduced following interferon administration (with bordering statistical significance because of the small sample size and the relatively short follow up) when compared to the rate of recurrence among Swedish historical controls or, for that matter, the generally observed rate of recurrence.

Emil Frei, Director of the Sidney Farber Cancer Center in Boston, then observed that it is not yet clear how the Scandinavian results will compare to those obtainable with recent effective chemotherapy, but that interferon's lack of toxicity could be considered a distinct advantage if it were to have clinical usefulness. It was agreed that the clinical trials in osteogenic sarcoma should be expanded.

The implication of this is that more human interferon must be produced.

A session of the workshop concerned with new methods of interferon production indicated that this expansion of production can be achieved by the application of modern technologies in large scale cell culture. The interferon so produced would still be insufficient for wide use. Thus biochemists must pursue human interferon's purification, sequencing and, ultimately, synthesis,

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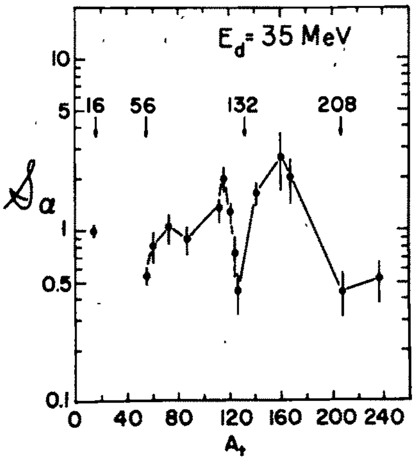
Alpha-transfer reactions

from P E. Hodgson

THERE is much current interest in alpha-transfer reactions (*Nature*, **252**, 270; 1974) because of the information they can give us about alpha-particle clustering in the nuclear surface There are many reactions that either remove an alpha particle from the nucleus or give an alpha particle to the nucleus, and among these the (d, ^{6}Li) reaction is particularly convenient because it is relatively easy to produce intense deuteron beams and to detect the emitted ^{6}Li ions

This reaction has recently been used by the Michigan group (Becchetti *et al*, *Phys. Rev Lett*, **34**, 225, 1975) to make a systematic study of alpha pickup in a range of nuclei from ^{12}C to ^{238}U They irradiated these nuclei with 35 MeV deuterons, and observed the ^{6}Li emitted at the angle corresponding to the maximum in the cross section for L=0 transfer. They found that the cross section falls rather steeply as the target mass increases (roughly as A_t^{-3}), with superposed fluctuations that are connected with the nuclear shell structure.

To study these variations in more detail, they calculated the cross section for alpha-particle pickup using the distorted wave theory This contains a factor S_α that is a measure of the probability of finding an alpha particle in the target nucleus. The resulting values of S_α, normalised to unity for ^{16}O, are shown as a function of A in the figure.



Alpha-particle spectroscopic factor S_α obtained from analyses of (d, ^{6}Li) reactions normalised to unity at ^{16}O and plotted as a function of target mass.

The fluctuations of S_α with A can be correlated with the nuclear shell structure in a very simple way. The minima correspond to nuclei with closed neutron or proton shells, and the maxima correspond to nuclei with open shells. Thus the maximum around $A=110$ corresponds to open neutron shells and that around 160 to open neutron and proton shells.

These results are closely parallel to those found by Milazzo-Colli and Braga-Marcazzan in their studies of (p, α) and (n, α) reactions (*Nature*, **245**, 12; 1973), and of alpha decay. The probability of alpha emission is given by the product of the probability of finding an alpha particle on the nuclear surface and the probability that it can penetrate through the potential barrier of the emitting nucleus. Analysis of data on many spontaneous alpha decays gave the variation of the alpha preformation probability with A (Bonetti and Milazzo-Colli, *Phys. Lett.*, **49B**, 17; 1974). Their result was similar to that shown in the figure and in particular showed a marked minimum in the region of the doubly closed shell nucleus ^{208}Pb.

Taken together, these results provide useful additional evidence concerning the probability of finding alpha particles on the nuclear surface.

which would be also greatly facilitated if more human interferon were available.

The workshop ended with a consensus that a broad-based international collaborative programme of laboratory and clinical work should be intensified, aimed first at establishing whether interferon has anti-tumour potential in osteogenic sarcoma, and then at exploiting its whole range of clinical potential.

Neglected research and the power of bureaucracy

from Kenneth Mellanby

The Council for Science and Society held an open forum on "Neglected Research and Social Priorities" in London on May 3, in order to identify neglected fields of research which might be of value to man and to society.

MAGNUS PYKE described the various actions the government had taken during and after the last war, with the intention of improving the health of Britain's population. He pointed out that the government was still insisting on considerable expenditure to fortify bread with calcium and vitamins, though no research had been done to find out whether these additives were actually doing good. We do not even know to what extent the improvement in health during the war was due to higher wages or to governmental benevolence.

Energy was the concern of Walt Patterson of Friends of the Earth. He believed that the government lacked a proper energy policy, and that more work should be done on such topics as wind, solar energy, bioconversion, advanced coal extraction technology and less on nuclear problems. The next speaker, Colin Tudge, was also primarily political. His topic was "What value is agricultural research?", and his conclusion was, "very little". His main concern was that most research supports western capitalism, and that the implementation of a Marxist system could produce better results and make most of this research unnecessary. He alleged that research on tropical crops of value to the third world was starved. This conclusion was not borne out by a report I received through the post on the same day, listing the lavish expenditure of the International Institute of Tropical Agriculture at Ibadan, Nigeria, where ambitious projects on yams, sweet potatoes, cassava, maize and tropical soil conservation are in progress

John Williamson of Guy's Hospital wanted more research into medical efficiency. He deplored the lack of effort given to common complaints like acne, cystitis and asthma, and on comparisons between self-treatment and that given by skilled clinicians. Nigel Calder, who spoke as a concerned journalist, was worried that the damage done by Lysenko to Russian genetics might be paralleled by the scope for obtuse though powerful scientists to block the supply of both public and private funds to fields of which "the establishment" disapproved. He cited the case of Professor Lamb's Climatology Unit in the University of East Anglia, which, even after prolonged negotiations failed to obtain support from any of the research councils; fortunately, in the event, it has been temporarily saved by private foundations. Calder compared unfavourably the many millions spent on meteorology, where the largest and most expensive computers have possibly made marginal improvements in the two and three day weather forecasts while, in Britain, climatology is almost totally neglected.

Eve Goss described, from her personal experience, the difficulty of the disabled in obtaining help from the authorities, and suggested that more research on how the old and disabled may be integrated into the community was desirable.

The open discussion was introduced by Colin Fisher of the Pye Charitable Trust, who listed a series of neglected areas, and tried to explain the reasons for this neglect. These included laziness and elitism in research workers themselves, and conservatism in the technocrats. He queried the wisdom of allowing the financing agencies to have so much power in stimulating research in their chosen fields. He showed how some of the private foundations may rely on the same advisors as the governmental organisations, so that the same dogmas are perpetuated He made a plea for a more agnostic approach, and for at least some support for whimsical problems.

H. H. Lamb explained the problems, largely financial, in starting new work in Britain. He gave chapter and verse for instances of totalitarianism in laboratories where workers were compelled to study projects they knew would prove unproductive, and were forbidden to follow lines which, later, proved to be important. He thought that the provision of some funds from private sources, from industry and from American and international sources was some insurance against the tyranny of the establishment.

Various speakers then described specific topics; there was some interest in Tony Benn's suggestions (made when he was a member of the government in 1966) that some research fund should be available to trade unions and consumer groups.

The forum did not reveal any vas fields where profitable work was needed, and where neglect was total It did reveal concern at the growing power of bureaucracy, and a dange that the original but unorthodo worker was finding it increasingly difficult to get support. To me the mos worrying cases were where, in some universities and research institutes workers were actually forbidden to tackle problems even when no extra funds were needed

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Contact between photoreceptors

from a Correspondent

THE idea that each vertebrate photoreceptor acts independently of other cells has long been explicit in accounts of visual function Yet for over a decade there has been anatomical evidence for connections between receptor cells (Sjostrand, *J. ultrastruct. Res.*, **2**, 122–170; 1958). In the last ten years, intracellular recording methods have advanced to the point where a full study has been made of turtle receptors; in particular, the electrical responses of cones have been modelled and a description has been made of local effects transmitted from other cones, and of a distant antagonistic effect mediated by horizontal cells. It seems that colour and pattern processing, and adaptation phenomena, begin at the most peripheral level in the visual pathway (Baylor, Fuortes, and O'Bryan, *J. Physiol., Lond.*, **214**, 265–294; 1971; Baylor, Hodgkin, and Lamb, *J. Physiol., Lond,.* **242**, 759–791; 1974).

Physiological evidence has also been presented that rod receptors are extensively coupled (Fain, *Science*, **187**, 838–841; 1975; Schwartz, *J. Physiol., Lond.*, **246**, 617–638; 1975). In addition, J. E. Dowling has described evidence (at the April meeting of the British Photobiological Society) of extensive 'fin-contacts' between rod receptors at the level of the outer limiting membrane. It seems that functional coupling between neighbouring rods may be used to signal single quantal absorbtions in the face of the various sources of receptor and transmission noise. Though the functional significance of the different varieties of inter-receptor contacts remains to be studied, it is clear that they have an important effect, and that our ideas of receptor excitation have undergone a brisk change.

articles

Salt floors to geosynclines

David J. J. Kinsman

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I propose that if young rift oceans (proto-oceans) are commonly the site of large scale evaporite deposition and if the sedimentary prisms of rifted margins are correctly identified as eugeosynclines, then it follows that geosynclines should commonly be floored by evaporites.

UNTIL a decade ago the linear belts of thick sedimentary rocks, commonly referred to as eugeosynclinal deposits, were generally thought to have accumulated within the craton on subsided sialic, or continental type crust. Hess[1] and Dietz[2,3] suggested that eugeosynclines were initially ensimatic and only later in their history were welded on to and into the continental craton. In the context of seafloor spreading Dietz proposed that the prism of sediments which accumulated along a rifted or trailing continental margin represented the early phases of eugeosynclinal accumulation. Deposition occurred partly on the thinned and submerged edge of the continent (the continental slope) but mostly on the adjacent oceanic crust to form the continental rise. Thus a relatively long, tectonically quiet stage of sediment accumulation, representing the trailing history of a continental margin, precedes the more tectonically active stage when the margin uncouples and oceanic crust is subducted. Most trailing continental margins can be envisaged as having undergone a history of: (1) initial intra-continental plate rupture, with formation of a new plate boundary; (2) continued seafloor spreading with addition of oceanic crust adjacent to the continental margin, moving the margin to an intraplate position; and (3) later uncoupling and new plate boundary inception between continental and oceanic crust at the continental margin. The maximum ages of trailing continental margins and of ocean floors suggest that the tectonically quiet stage of sediment accumulation may be as long as 150–200 Myr, but this value probably depends on long term, average, global spreading rates.

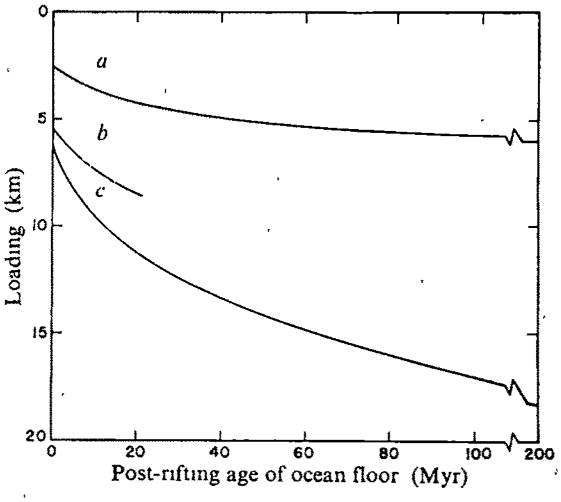
Sclater and Francheteau[4] have demonstrated that most new oceanic crust generated at spreading ridges starts life between 2 and 3 km below sea level and subsequently follows a time dependent subsidence, related to heat loss and thermal contraction. An average depth–age curve for oceanic crust is shown in Fig. 1. This curve describes the depth history of sea floor when loaded with water and a negligibly thin veneer of sediment. Hot spot or plume generated[5] sea floor may, however, be formed at shallower depths or even subaerially, although it also apparently follows a similar time dependent subsidence[6,7]. Sleep[8] has used a similar thermal model to explain the subsidence rates of North Atlantic rifted continental margins and Kinsman[6] has also used this thermal model to describe the uplift, rifting and subsidence history of rifted continental margins. Assuming isostasy at all times and combining sediment density–depth data with the thermal subsidence model, it is possible to determine the maximum marine sediment loading which can be accommodated on rifted continental margin of various crustal thicknesses and on ocean floor, both with reference to their post-rifting age[6]. The maximum loading by marine sediments increases seaward off the continental margin as continental crustal thicknesses decrease and is greatest on oceanic crust of normal thickness. The maximum marine sediment loading of oceanic crust increases with post-rifting age of the crust but is predicted using this loading model to be close to 16–18 km for the range of average sediment densities (Fig. 1). Along trailing continental margins thicknesses of this order are found under several of the world's largest deltas where sedimentation has now definitely stepped well clear of the continental margin and oceanic crust is being deeply buried (examples include Niger delta[9]—14 km; Mississippi delta[10]—16–17 km; Ganges delta[11]—16–17 km). A considerable length of the Atlantic continental margin of North America is mantled by a sediment prism 10–12 km thick[10]. Reconstructed ancient geosynclinal sediment thicknesses are also commonly in this range[12].

Thus the sediment loading capacities of rifted continental margins, the maximum age of rifted margins and the rate of delivery of sediment from the craton may all lead to massive sediment prisms being developed along rifted continental margins. The oldest oceanic crust and the continent–ocean transition zone, together with the earliest sediments deposited in the proto-ocean, typically become very deeply buried.

Continental margin and ocean floor evaporites

Bathymetric and geophysical surveys of certain continental slopes and rises and of adjacent deep oceanic areas give abundant evidence of the presence of diapiric structures. A variety of arguments suggests that most of these are evaporite

Fig. 1 Subsidence histories of ocean floor as a function of post-rifting age. (*a*), Increase in water loading of oceanic crust as a function of post-rifting age (modified from Sclater and Francheteau[4]); (*b*), maximum ocean floor loading with halite as a function of post-rifting age; (*c*), maximum, isostatic, marine sediment loading of ocean floor as a function of post-rifting age (from Kinsman[6]).



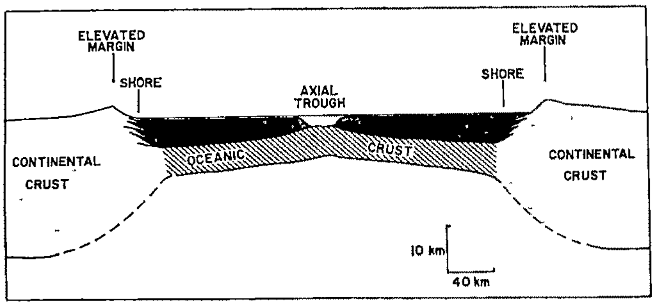


Fig. 2 Schematic depicting proto-ocean evaporites in the southern Red Sea (adapted from data in Hutchinson and Engels[16] and Whitmarsh *et al.*[17]). The upper 3–4 km of the evaporites are proved by drilling, the lower section from seismic information; data available mainly from the western margin of the Red Sea.

diapirs rather than igneous intrusions or mud diapirs[13]. In a few instances drilling has recovered typical salt dome cap rock, as on the Sigsbee Knolls of the central Gulf of Mexico[14]. In other instances pore waters above the diapiric structures have proved to be very saline, indicating the deeper presence of halite[15].

Hutchinson and Engels[16] and Whitmarsh *et al.*[17] provide information on the Red Sea continental margin and proto-ocean evaporites; the marginal and proto-ocean evaporites from the South Atlantic have most recently been described by Pautot *et al.*[18]; the North Atlantic continental margin evaporite data have been summarised by Emery and Uchupi[10]. These three oceans are all rift oceans in various stages of development; the Red Sea is a proto-ocean in an early stage of development (~20 Myr), the South Atlantic is a mature rift ocean (~100–120 Myr), and the North Atlantic is entering its probable senescence as a rift ocean (~160–200 Myr). In all three oceans the evaporites are recorded in marginal basins, down the continental slope and on oceanic crust. For example, in the South Atlantic diapirs are reported seaward of the Niger Delta[19] in an area which is surely underlain by oceanic crust as it comprises an overlap zone in all pre-rift reconstructions. The lateral extent of the evaporites is such that salt flowage or creep seaward down the continental slope and across the adjacent ocean floor seems a most unlikely explanation of the diapirs overlying oceanic crust. The detailed pattern of evaporite deposition in and along the margins of the proto-ocean may have been very complex. For example, local evaporite formation may proceed in marginal grabens or basins such as the 1-km thick Pleistocene evaporites of the Danakil Depression[16] which were formed while the main Red Sea proto-ocean was in a non-evaporite mode. At other times the entire proto-ocean may become partially or completely isolated and the brine–air interface may exist anywhere between world ocean level and the floor of the proto-ocean basin 2–3 km below. The evaporites may therefore show the characteristics of shallow water or even subaerial sabkha facies[20], or be well layered deep brine basin deposits.

The presence of thick evaporites in rift valley basins and in proto-oceans suggests that times of initiation of new continental rifting and proto-ocean formation may be major evaporite formation periods. The widths of these continental margin evaporite belts, when combined with probable spreading rates, suggest that evaporite formation occurs during the initial 10–20 Myr of spreading[6,21]. The distribution of the Red Sea evaporite thicknesses and the age of the Red Sea conform fairly closely to the predicted maximum salt loading curve for oceanic crust (Fig. 1). The evaporites are about 3 km thick immediately west of the present axial trough (Fig. 2) and thicken westward to a maximum of about 7 km (ref. 16). This landward thickening suggests that the evaporites are overlying oceanic crust which increases in age westward. The evaporite and sediment section thins again westward of the maximum 7 km evaporite zone, presumably because of the gradual westward thickening of continental crust (Fig. 2).

If the future history of the Red Sea continental margins is to be similar to that recorded for most ancient rifted continental margins, subsidence will depress the entire belt of thinned continental crustal margin, the adjacent coupled oceanic crust and the overlying evaporite body. Normal marine sediments more than 10 km thick, will probably be deposited on top of the evaporites. The occurrence of continental margin evaporites in the South Atlantic (~120–110 Myr) and North Atlantic (~200–160 Myr) can be reasonably understood if these oceans underwent an early history similar to that of the Red Sea, with thick evaporites laid down over the entire continental margin and oldest ocean floor belt (Fig. 3).

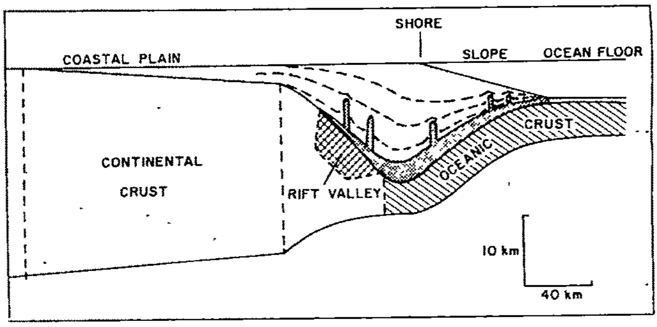
Widths of the continental margin evaporites vary greatly and along some local lengths of margin evaporites may be absent. Evaporites are often present in minor rift basins but are absent over local structural highs. Offshore data are generally too sparse to be certain of the detailed evaporite distribution. But in the Red Sea the belt of evaporites is up to 100 km wide along the south-west margin. In the South Atlantic the belt of evaporites in the coastal basins and extending down the continental slope to the abyssal plain is 50–300 km wide[18]; similar widths are reported off the Brazilian coast[22]. In the North Atlantic, diapirs have been reported 1,300 km off the north-west African coast[23], but these are not yet proven to be evaporites. The Gulf of Mexico evaporites also cover a very wide area. Thus the widths of rifted continental margin evaporite belts are such that most of the future eugeosynclinal sediment prism will be deposited on top evaporitic sediments. Only along continental margins where sediment input rates are very high may the width of the evaporite belt be overstepped by the thick prism of sediments.

Frequency of evaporite formation

How frequently are evaporites formed in proto-oceans? To form an evaporite deposit two independent requirements must be met.

First, the proto-ocean must be located in a climatic belt of net evaporation. Present-day marine evaporites are largely restricted to the latitudes 30–35° N or S of the Equator, although a few occurrences extend to 40° N or S. Smith *et al.*[24] and Drewry *et al.*[25] have published a series of maps showing the likely predrift configurations of the continents and their palaeolatitudinal positions for various past geological periods. One of their major conclusions, based on the distribution of marine evaporites and other climatically sensitive sediments is that since at least late Mesozoic times the high pressure subtropical belts of net evaporation have had approximately their present-day width and have been fairly stable features of the atmospheric circulation. It seems reasonable, therefore, to assume that nearly all evaporites over the past 200 Myr have formed between the latitudes 35°N and 35°S.

Fig. 3 Schematic depicting proto-ocean evaporites deeply buried at the base of a trailing continental margin sediment prism (modified from Fig. 10 of ref 6). This section is similar in most respects to the probable north–south section across the Mississippi Delta and northern Gulf of Mexico.



Second, proto-oceans within the climatic belt of net evaporation must develop sufficient physical restriction to seawater influx. The restriction requirement is extremely demanding as the seawater influx must be less than the total evaporation, and this latter parameter is climatically constrained to values of less than 2–3 m yr^{-1}. The barriers restricting seawater influx to proto-oceans comprise the elevated margins of the rifted continents along the length of the proto-ocean, together with transverse barriers which may be continental fragments faulted off the main continental margin, continental segments where rifting is transcurrent, or volcanic islands over deep mantle plume sites[6,21].

The maps of Smith *et al.*[19] together with additional data on age of rifting in various localities and on presence or absence of evaporites along the rifted continental margins have been used to generate Table 1, *a* and *b*.

Of the rift basins and proto-oceans in which evaporites were formed only the Tanzania example is at a latitude higher than 30° (Table 1*a*). The total axial length of proto-ocean generated over the past 200 Myr between latitudes 35°N and 35°S is approximately 11,000 km and in 9,000 km evaporites were formed on a massive scale (1–7 km thick). Thus, rather than being an unusual phenomenon, evaporites are the expected and predictable sediments to form in low latitude proto-oceans during their initial 10–20 Myr of development. There is an 80–85% probability of the physical barrier requirement being met and this is a purely geological parameter independent of the climatic requirements.

A rough estimate of the proto-ocean formed at latitudes higher than 35° over the past 200 Myr is 14,500 km (Table 1*b*). During this period of 200 Myr nearly 35% of the total proto-ocean length has been deeply mantled by an early deposit of evaporites. In other words, 35% of potential eugeosynclinal floors formed over the past 200 Myr are underlain by thick and laterally extensive evaporite deposits.

A corollary of the frequency with which low latitude proto-oceans develop a physical restriction sufficient for them to become evaporitic is that in latitudes of net precipitation, the majority (~80%) of proto-oceans should have been brackish during their initial 10–20 Myr of post rifting development.

The climatic belt in which evaporites may form comprises approximately half the area of the Earth. If the frequency of achievement of physical restriction to seawater influx into proto-oceans is about 80% and if proto-oceans have been generated throughout the Earth's history with no latitudinal bias, then about 40% of all rifted continental margins should have been mantled with evaporites. If all eugeosynclines have gone through an early trailing continental margin stage then 40% of the axial length of all eugeosynclinal floors should have been mantled by evaporites.

Consequences of proto-ocean evaporite formation

There are several interesting consequences to the proposal that evaporites very commonly form in proto-oceans yet later line the floors of geosynclines:

Evaporite masses formed in proto-oceans are commonly very large (500–4,000 km long, 100–600 km wide and 1–7 km thick) and the period of formation is relatively short (< 10–20 Myr). Compared with the rate of input of Na^+ and Cl^- into the ocean[26], the evaporite withdrawal of NaCl as halite is essentially instantaneous and will produce a salinity decrease of the world ocean. Estimates of NaCl stored in evaporites are certainly too small and are in need of drastic upward revision (my unpublished work); when these data are available reasonable estimates can be made of the evaporite controlled salinity excursions of the world ocean. If these salinity excursions are large enough to be reflected in the biotic record, then a link may be established between times of large scale rifting and inception of seafloor spreading and biotic crises, through the evaporite formation mechanism.

The deep burial of evaporites beneath thick sediment prisms opens an interesting avenue to the problem of the sodium cycle. The weathering of crustal silicate rocks releases sodium to the ocean and forms a sodium depleted sediment. With passage of time, considerable burial, or moderate temperature increase, there is, however, little or no marked accession of sodium to sediments[27,28]. Even low grade metamorphosed sedimentary rocks such as mica schists are still sodium depleted. Higher grade feldspar gneisses, however, have sodium values typical of average continental crustal rock[28]. These relationships suggest that halite dominated evaporites are subducted together with

Table 1 Proto-oceans and continental margins

	Proto-Ocean/Continental Margin*	Rifting/evaporite age (Myr)†	Palaeolatitude‡	Axial length (km)§
a	With evaporites			
	Red Sea + Danakil Depression	25–0 (U Oligo–Rec)	5°N–30°N	2,000
	South Atlantic	120–110 (Aptian–L. Albian)	10°S–30°S	2,500
	Tanzania	220–170 (U. Trias–M. Jur.)	35°S	500
	N Atlantic + Gulf of Mexico	200–160 (U. Trias–M. Jur.)	5°N–25°N	4,000
			Total	9,000
b	Without evaporites			
	Gulf of California	~5 (Plio.–Rec.)	20°N–30°N	1,200
	Gulf of Aden	15–10 (Mio.–Plio.)	5°N–10°N	800
			(Low latitude subtotal)	2,000
	Australia–Antarctica	~50 (Eoc.)	60°S–65°S	2,500
	N. Atlantic–Norway, Greenland	~60 (U. Palaeoc.–L. Eoc)	45°N–60°N	2,500
	Baffin Bay	~60 (U. Palaeoc –L. Eoc.)	45°N–60°N	1,500
	Australia–New Zealand	80–60 (U. Cret.–Palaeoc.)	50°S–70°S	1,000
	India–Antarctica	~125 (L Cret.)	40°S–50°S	1,500
	West Australia–India	~125 (L. Cret.)	40°S–50°S	1,000
	Africa–Madagascar Madagascar–India	~140 (U. Jur.–L. Cret.)	35°–50°S	3,000
	NW Australia–?	~150 (U. Jur.)	40°S–50°S	1,500
			Total	16,500

* Proto-oceans are identified where possible by use of the ocean name, or by use of the two bounding rifted continental margins (for example, Africa–Madagascar); where only one rifted margin name appears, we have no evidence of the opposing margin. The Tanzania evaporites were apparently formed in a marginal rift graben; no evaporites were formed in the associated proto-ocean.

† Rifting/evaporite age data are from refs 7, 10, 17, 18, 31–34.

‡ Palaeolatitude data are derived from using age data of previously mentioned authors in conjunction with maps of Smith *et al*[24]. The large atitudinal range of Africa–Madagascar–India in part reflects the uncertainty of the palaeoposition of Madagascar relative to the African continent.

§ Axial length refers to proto-ocean length (about twice this length of rifted continental margin has been formed over the past 200 Myr).

sodium depleted sediments and that sodium metasomatism occurs during metamorphism. The halite will penetrate the sediment mass partly in the anhydrous state as diapirs and partly in solution, being dissolved and mobilised by remnant pore water and structural water removed from hydrated minerals during dehydration reactions. A sediment column 15 km thick would need to react with only about 200 m of halite to restore its sodium content to that of average crustal rock. Recent reports of NaCl brines in fluid inclusions from high grade metamorphic rocks may support this proposal[29]. One might predict that if such solutions gained their Na^+ and Cl^- from halite dissolution then the Br^-/Cl^- ratios should be much lower than that of seawater.

Halite deposits high on the continental margin presumably will not be subducted. These could form a surface of detachment enabling some of the compressed sediment prism to ride more readily up on to the continental margin.

The continental slopes are becoming of economic interest. Many of the world's hydrocarbon reservoirs are sealed by evaporites because they have essentially zero porosity and permeability. Are there any likely hydrocarbon source beds beneath the extensive evaporites of early rift oceans? If the evaporites form so early in the history of the ocean one might surmise the probability to be small. But the immediately pre-evaporite phase of restriction, leading to hypersalinity is one in which stable stratification of water bodies may be expected. In addition, dissolved molecular oxygen in the seawater brines is low because of relatively high water temperatures (20–30 °C) and increasing salinity[30]. As yet, we know little about organic productivity in evaporite basins but it seems likely that much of the organic matter which is produced will be sedimented and not oxidised. There is a fair possibility that early rift oceans with evaporites could have had organic rich sediments accumulate within them previous to evaporite deposition. Hydrocarbons generated from these sediments would be sealed beneath the overlying evaporites and might occur in marginal reservoir facies along the continental margin.

I thank M. Arthur, K. S. Deffeyes, A. G. Fischer, R. B. Hargraves, F. B. Van Houten and R. Yuretich for comments. These studies were largely completed during my tenure of a Guggenheim Memorial Fellowship.

Received March 17; accepted April 14, 1975.

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Induction of increased calcium uptake in mouse T lymphocytes by concanavalin A and its modulation by cyclic nucleotides

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The binding of concanavalin A to T but not B mouse spleen lymphocytes increases Ca^{2+} uptake in these cells which is measurable by 45 s and complete by 1 min. Dibutyryl cyclic AMP, but not sodium azide inhibits induced Ca^{2+} uptake, whereas dibutyryl cyclic GMP enhances it. B cell mitogens do not cause a similar Ca^{2+} uptake in mouse B lymphocytes. The induction of increased Ca^{2+} uptake by T cells is discussed in terms of gated membrane channels for Ca^{2+}.

CELL surface receptors receive and transduce a variety of environmental signals. The binding of such a signalling ligand (or 'first messenger') induces a change in the receptor, initiating the next event(s) by which information is carried across the membrane into the cell interior. The cyclic nucleotides, cyclic AMP and cyclic GMP, which are regulated by specific membrane bound enzymes (cyclases), have received much attention as 'second messengers' in the signalling of cellular activity. It is clear, however, that in some situations it is the opening of ion permeability pathways through membranes which serves this function by allowing the transfer of selected ions, such as Na^+ and Ca^{2+}, down their concentration gradients into the cytosol. Thus, Ca^{2+} is the second messenger in excitation–contraction coupling in skeletal muscle[1] and probably plays a similar role in the 'stimulus-secretion coupling' of many triggered secretory systems[2]. There is increasing indirect evidence that is consistent with a role for Ca^{2+} as a transducing mediator in more prolonged responses, such as the lectin-induced proliferation of lymphocytes. These responses are dependent on the presence of Ca^{2+} in the extracellular medium[3–5] and an increased influx of radioactive $^{45}Ca^{2+}$ has been

Table 1 Measuring and calculating $^{45}Ca^{2+}$ uptake*

Mitogen	Time of incubation (min)	^3H_2O (c.p.m.)	$^{45}Ca^{2+}$ (c.p.m.)	$^{45}Ca^{2+}/^3H_2O$	$^{45}Ca^{2+}$‡ uptake	Corrected§ ^3H_2O (c.p.m.)	Corrected‖ $^{45}Ca^{2+}$ (c.p.m.)	Corrected $^{45}Ca^{2+}/^3H_2O$	Corrected $^{45}Ca^{2+}$ uptake
None†	0	1,478±62	760± 33	0.514±0.01	—	—	—	—	—
None	15	1,325±80	948± 34	0.719±0.03	1.0 ±0.036	869	188	0.216	1.0
Con A ($3 \mu g\ ml^{-1}$)	15	1,889±15	2,110± 23	1.117±0.003	1.55±0.004	1,433	1,350	0.942	4.36
A23187 (2×10^{-5} M)	15	1,446±92	2,993±143	2.066±0.05	2.87±0.07	990	2,233	2.255	10.49

* Results of a typical experiment done in triplicate, expressed as mean ± s.d.; cells, mitogens and isotopes incubated as in text, or as indicated.

† Chilled $^{45}Ca^{2+}$ and ^3H_2O added to cells at 4 °C and centrifuged immediately; $^{45}Ca^{2+}$ counts are mainly due to exchange with surface-bound cations and radioactivity in entrained water space (see text).

‡ $^{45}Ca^{2+}$ uptake is calculated by dividing the $^{45}Ca^{2+}/^3H_2O$ value of the sample by that of the control cells incubated with MEM alone, so that control ratio is normalised to 1.0. The increase above 1.0 is a measure of the mitogen-induced $^{45}Ca^{2+}$ uptake.

§ Corrected by subtracting the 0.15% of the ^3H_2O load (= 456 c.p.m. in this experiment) which is in the entrained water space (see text).

‖ Corrected by subtracting the $^{45}Ca^{2+}$ counts at time 0 at 4 °C (= 760 c.p.m.).

reported to occur within minutes of PHA binding to human blood lymphocytes[6–8]. It has not so far been demonstrated, however, that the cells showing increased permeability for Ca^{2+} are those which are later induced to divide. Recently, pig[9] and human[10] lymphocytes have been induced to synthesise DNA by application of the divalent cation-carrier molecule (ionophore) A23187, in a manner dependent on the presence of extracellular Ca^{2+}. This suggests that the influx of Ca^{2+} may be a sufficient signal for proliferation. So far, rodent lymphocytes have not been reported to respond to the ionophore, and except for one preliminary experiment[7], there are no substantive reports of Ca^{2+} influx occurring as a consequence of the binding of lectins to these cells. Here we report that concanavalin A (con A) induces increased $^{45}Ca^{2+}$ uptake in mouse T, but not B lymphocytes within 45 s of the addition of lectin. This effect is short lived, and is inhibited by high concentrations of dibutyryl cyclic AMP but not by sodium azide, and it is enhanced by dibutyryl cyclic GMP. In addition, we show that two B lymphocyte mitogens do not cause a measurable increase in $^{45}Ca^{2+}$ in B cells. These findings raise important questions about the possible role of gated Ca^{2+} channels in ligand-induced lymphocyte proliferation.

Measuring $^{45}Ca^{2+}$ uptake

BALB/c mouse spleens were teased in Eagle's medium (containing 1.8 mM Ca^{2+}), buffered with HEPES 20 mM (instead of HCO_3^-) to *p*H 7.2 (MEM), passed over a short column of glass wool to remove debris and cell clumps, treated with Tris-buffered ammonium chloride *p*H 7.2, for 10 min at room temperature to lyse red cells[11], washed twice, and adjusted to 20×10^6–30×10^6 cells ml^{-1}. Cells (80 μl) were incubated with 10 μl of con A (Miles-Yeda) or MEM, and 10 μl of a mixture of $^{45}CaCl_2$ (1 μCi) and ^3H_2O (5 μCi). The equilibration of water across phospholipid and biological membranes at 37 °C is very rapid[12], allowing the uptake of tritiated water in these experiments to serve as an indicator of cell number. After 15 min at 37 °C, the cells were separated from the radioactive loading solution by centrifugation at 12,000*g* for 3 min through a water impermeable barrier of silicone oil (Versilube F50) into 20 μl of 98% formic acid. Polypropylene microcentrifuge tubes (Beckman PRO-22) and a Beckman 152 Microfuge were used for this purpose. After centrifugation, the contents of the tubes were frozen by immersion in liquid nitrogen, and the bottom 1 cm, containing the cells now dissolved in formic acid, was amputated. This was placed in 3 ml of Instagel (Packard) and counted in a Packard Tricarb liquid scintillation spectrometer. The ratio of $^{45}Ca/^3H$ counts is proportional to the uptake of $^{45}Ca^{2+}$ per cell.

When ^{51}Cr-labelled spleen cells were handled in this way, 85–90% of the radioactivity was transferred into the formic acid, indicating that the great majority of the cells were centrifuged through the silicone oil. By adding ^3H-sucrose to the cell suspensions as a marker of entrained water space it was demonstrated that 0.15% of the volume of the loading solution was transferred through the silicone layer with 3×10^6 cells (that is 0.15 μl). When chilled $^{45}Ca^{2+}$ was added to cells incubated in MEM at 4 °C and immediately centrifuged, a relatively large uptake of activity was consistently seen, amounting to about 0.4% of the total $^{45}Ca^{2+}$ in the loading solution, but representing about 60–80% of the $^{45}Ca^{2+}$ uptake occurring when cells were incubated for 15 min at 37 °C (Table 1). Thus the major part of the $^{45}Ca^{2+}$ associated with the cells in these experiments probably represented rapid exchange of the labelled material with the cations adsorbed to the cell surface and was not related to membrane transport.

Table 2 Effect of mitogens on $^{45}Ca^{2+}$ uptake in BALB/c spleen lymphocytes, human RBC and mouse lymphoma cells*

Cells	Mitogen†	Concentration ($\mu g\ ml^{-1}$)	No. of experiments	Uncorrected‡ $^{45}Ca^{2+}$ uptake
Spleen	None	—	—	1.0
Spleen	Con A	3	31	1.30±0.034
Spleen	PHA	4	7	1.22±0.034
Spleen	Pokeweed	15	1	1.39±0.052
Spleen	*Axinella*	10	3	1.00±0.012
Spleen	LPS	20	17	0.98±0.011
Spleen	A23187	2×10^{-5} M	17	3.09±0.156
Spleen	A23187	2×10^{-6} M	1	2.04±0.012
RBC§	None	—	—	1.0
RBC	Con A	4	1	0.95±0.004
RBC	LPS	20	1	0.90±0.001
RBC	A23187	2×10^{-5} M	3	13.02±0.304
WEHI-22‖	None	—	2	1.0
WEHI-22	Con A	3	2	0.98±0.020
WEHI-22	Con A	50	2	1.55±0.061
WEHI-22	Con A	100	2	1.95±0.066
EL-4¶	None	—	1	1.0
EL-4	Con A	3	1	1.01±0.031

* Cells, isotopes and mitogens were incubated at 37 °C for 15 min; each experiment was done in triplicate.

† Con A = 3× crystallised concanavalin A (Miles-Yeda); PHA = purified *P. vulgaris* phytohaemagglutinin (Wellcome); pokeweed = pokeweed (*Phytolacca americana*); mitogen was a gift from Dr G. Janossy and was prepared as described by Borjeson *et al.*[14]; LPS = lipopolysaccharide from *salmonella marcenscens* (Difco); *Axinella* = *Axinella polyploides* agglutinin, was a gift from Dr M. Crumpton, and was prepared as previously described[15]; A23187 was supplied by Dr R. L. Hamill (Eli Lilly). Con A, PHA, pokeweed, and LPS were used at concentrations which gave maximum DNA synthetic responses in spleen cells at 72 h.

‡ Determined as in Table 1 and expressed as mean ± s.d. of the means of each experimental triplicate. Where only 1 experiment was done, the mean ± s.d. of the triplicate is given. Positive responses are underlined.

§ Human RBC were collected in heparinised MEM, washed twice and used at about a 5% suspension.

‖ WEHI-22 is a cultured cell line from an irradiation-induced BALB/c θ^+ lymphoma[17].

¶ EL-4 is a cultured cell line from a chemically-induced C57B1 θ^+ thymoma[1].

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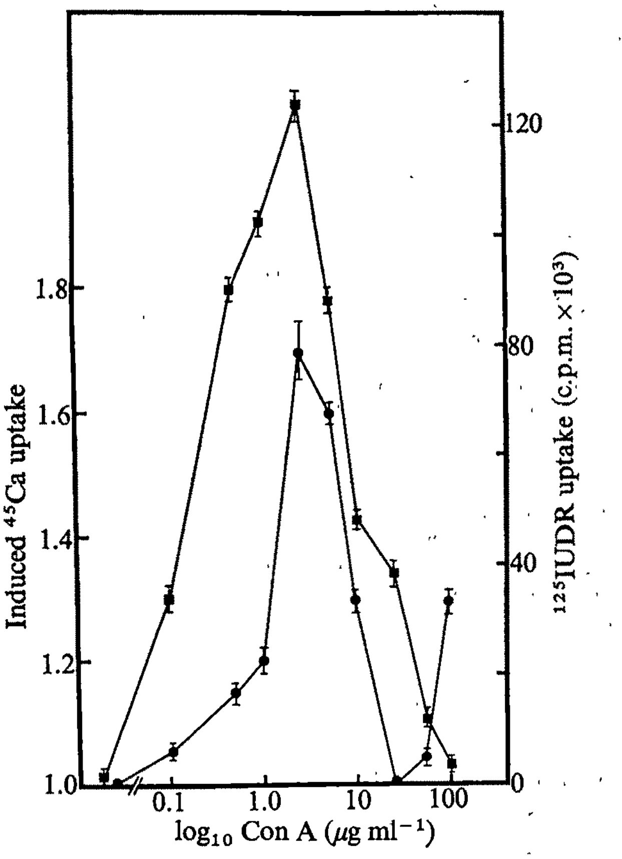

Fig. 1 Dose responses of uncorrected Con A-induced $^{45}Ca^{2+}$ uptake (●) measured at 15 min (as in Table 1) and DNA synthesis (■) measured by ^{125}IUDR incorporation at 72 h. The same BALB/c spleen suspension at 30×10^6 cells ml^{-1} in RPMI-1640 (buffered with bicarbonate) medium plus 10% foetal calf serum was used for both assays. For ^{125}IUDR assay, 200 μl of cells were incubated with con A for 60 h in Limbro Microtest plates at which time 1 μCi in ^{125}IUDR was added to each well for another 12 h. The cells were collected on to Whatman GF/A filter paper, washed with water and counted in a Wallac γ-counter. In both assays, results are expressed as means ± s.d. of triplicates.

$^{45}Ca^{2+}$ uptake increased by T cell mitogens

Spleen cells exposed to appropriate concentrations of known T lymphocyte mitogens[13], including con A, phytohaemagglutinin (PHA) and pokeweed mitogen, showed measurable increases in $^{45}Ca^{2+}$ uptake compared with cells incubated in MEM (Tables 1 and 2). When $^{45}Ca^{2+}/^{3}H_2O$ ratios were compared, these increases varied between 10 and 80%. If the 60–80% of the $^{45}Ca^{2+}$ counts present at the cell surface and within the entrained water space were subtracted, however, the increases were 150–500% (Table 1). Except for Table 1, all of the data are presented as uncorrected ratios.

Spleen cells treated with various concentrations of lipopolysaccharide from *Salmonella marcenscens* (LPS)[13] or *Axinella polyploides* agglutinin[15], both of which are known not to be mitogenic for mouse T cells, failed to show increased $^{45}Ca^{2+}$ uptake. A23187, the divalent cation ionophore[16], permitted spleen cells to increase their $^{45}Ca^{2+}/^{3}H_2O$ ratio 2–4-fold (Table 2). Human red cells and cell lines derived from two θ+ mouse lymphomas, WEHI-22[17] and EL-4[18], did not show an increase in $^{45}Ca^{2+}$ uptake when exposed to mitogenic (for T cells) concentrations of con A, but WEHI-22 cells did when exposed to very high concentrations of the lectin (Table 2).

The dose responses for con A-induced $^{45}Ca^{2+}$ uptake and DNA synthesis (measured by the ^{125}IUDR incorporation method at 72 h) determined for the same spleen cell suspension in identical conditions of incubation are shown in Fig. 1. At the lower concentration end, the dose–response curves were very similar, with a sharp peak at about 3 μg ml^{-1}. Although no DNA synthesis was seen with concentrations of con A greater than 25 μg ml^{-1}, the uptake of $^{45}Ca^{2+}$ increased again at these high concentrations. Marked agglutination and sticking of the cells to the walls of the test tube occurred at these high concentrations of con A, making it difficult to interpret this second rise in $^{45}Ca^{2+}$ uptake. No appreciable agglutination of the spleen cells occurred at concentrations of con A ≤3 μg ml^{-1}.

Only T cells respond

Although soluble con A binds to all mouse spleen cells and the same number of molecules binds with equal avidity to both T and B lymphocytes[19], only T cells are stimulated to proliferate[13]. To find out if the con A-induced $^{45}Ca^{2+}$ uptake was also restricted to the T lymphocytes, spleen cells were treated with anti-θ C3H (anti-thy-1.1) antiserum and guinea pig complement to kill the T cells[20]; control cells were treated with normal mouse serum and complement. In both cases, dead cells were removed, following treatment, by a 5-min exposure to 0.05% trypsin at 37 °C. The control cells (containing 36% T cells as judged by direct immunofluorescence with highly specific fluorescein-conjugated anti-θ C3H[21]) showed a 30% increase in $^{45}Ca^{2+}$ uptake in the presence of con A, while the cells which had been treated with anti-θ antibody and complement (less than 1% T cells) failed to show any increment (Table 3). Spleen cells from congenitally hypothymic *nu/nu* (nude) mice, known to be markedly deficient in T cells[21], failed to show an increase in $^{45}Ca^{2+}$ uptake on exposure to mitogenic concentrations of con A, but did so at very high concentrations (Table 3). Thus the increase in the uptake of $^{45}Ca^{2+}$ at mitogenic concentrations of con A depended on the presence of T cells, whereas that seen at high concentrations (>10 μg ml^{-1}) did not.

Although mouse spleen B lymphocytes are induced to proliferate by bacterial lipopolysaccharide (LPS)[13], no increase

Table 3 Effect of mitogens on $^{45}Ca^{2+}$ uptake in T cell-depleted spleen cells

Cells	Treatment* of cells	Mitogen	Concentration (μg ml^{-1})	No of experiments	Uncorrected† $^{45}Ca^{2+}$ uptake
BALB/c spleen	anti-θ	none	—	—	—
BALB/c spleen	anti-θ	Con A	0.5	2	0.95±0.006
BALB/c spleen	anti-θ	Con A	3.3	2	0.99±0.011
BALB/c spleen	anti-θ	Con A	5.0	2	0.97±0.027
BALB/c spleen	NMS	none	—	—	—
BALB/c spleen	NMS	Con A	0.5	2	1.09±0.017
BALB/c spleen	NMS	Con A	3.3	2	1.30±0.033
BALB/c spleen	NMS	Con A	5.0	2	1.22±0.025
nu/nu spleen‡	—	none	—	—	1.00
nu/nu spleen	—	Con A	0.5	6	1.01±0.02
nu/nu spleen	—	Con A	3.3	6	0.98±0.02
nu/nu spleen	—	Con A	5.0	6	0.98±0.013
nu/nu spleen	—	Con A	10.0	6	1.00±0.008
nu/nu spleen	—	Con A	50.0	3	1.55±0.11
nu/nu spleen	—	Con A	100.0	3	1.80±0.25
nu/nu spleen	—	LPS§	10.0	4	0.99±0.01
nu/nu spleen	—	LPS§	30.0	4	1.01±0.017
nu/nu spleen	—	LPS§	50.0	4	1.03±0.02
nu/nu spleen	—	Pokeweed§	5.0	3	0.96±0.025
nu/nu spleen	—	Pokeweed§	15.0	3	1.01±0.006
nu/nu spleen	—	Pokeweed§	25.0	3	0.96±0.015

* 200×10^6 cells were treated with 2 ml of anti-θ C3H or normal mouse serum (NMS) diluted 1:4 in MEM for 30 min at 37 °C, washed and incubated with 2 ml of guinea pig complement (absorbed with mouse spleen cells) at 1:4 for 30 min at 37 °C. After washing, cells were treated with 2 ml of 0.05% trypsin in MEM for 5 min at 37 °C to remove dead cells and washed twice. Anti-θ-treated cells contained 0.8% T cells as judged by direct immunofluorescence with anti-θ C3H-fluorescein, while NMS-treated cells contained 36% T cells. Cells were tested as described in text.

† Determined as in Table 1 and expressed as mean ± s.d. of each experimental triplicate. Positive responses are underlined.

‡ *nu/nu* mice were bred at the Clinical Research Centre, Harrow, and where supplied by Dr E. Simpson.

§ When used at these concentrations, these preparations stimulated *nu/nu* spleen cells to synthesise DNA (tested by Dr G. Janossy).

in $^{45}Ca^{2+}$ uptake could be detected when BALB/c or *nu/nu* spleen lymphocytes were treated with mitogenic concentrations of this B cell mitogen (Tables 2 and 3). Pokeweed mitogen, which stimulates both T and B mouse lymphocytes to proliferate[13], also failed to induce $^{45}Ca^{2+}$ uptake in *nu/nu* spleen cells (Table 3) but did so in BALB/c spleen cells (Table 2). LPS and pokeweed mitogen failed to cause an increase in $^{45}Ca^{2+}$ uptake in *nu/nu* spleen cells even when incubated with the cells and isotopes for 60 min, instead of the standard 15 min.

Con A-induced Ca^{2+} uptake is transient

When cells were incubated at 37 °C with a mitogenic concentration of con A, or with MEM, in the presence of $^{45}Ca^{2+}$, and then sampled at various times thereafter, a con A-induced increment in the uptake of the isotope was apparent at 45 s and maximal at 1 min (Fig. 2*a*). Although $^{45}Ca^{2+}$ continued to be taken up by both treated and untreated cells beyond this time the difference between the two remained unchanged for a further 4 h (data not shown). This was observed in 5 out of 5 experiments, done in various media, in the presence and absence of added foetal calf serum (10%). These results suggested that the con A-induced $^{45}Ca^{2+}$ uptake was of brief duration, and this interpretation was confirmed by experiments in which the $^{45}Ca^{2+}$ was added to the cells 5 min after the con A. These showed very little difference in $^{45}Ca^{2+}$ uptake compared with the control cells (Fig. 2*b*). In contrast, equal amounts of $^{45}Ca^{2+}$, added as a pulse, were taken up by red cells at any time following treatment with the ionophore A23187 (data not shown), indicating that this pathway remains open.

Influence of cyclic nucleotides

Many lymphocyte responses induced by antigens or lectins can be inhibited or enhanced by manipulations which increase intracellular cyclic AMP or cyclic GMP levels respectively (reviewed in ref. 22). Whitney and Sutherland found that dibutyryl cyclic AMP ± theophylline inhibited $^{45}Ca^{2+}$ uptake in human blood lymphocytes treated with PHA[7]. As can be seen in Fig. 3, treatment with dibutyryl cyclic AMP (10^{-2}M–10^{-4}M) caused a graded reduction (up to 89%) in the $^{45}Ca^{2+}$ uptake induced by mitogenic doses of con A added 30 min later. This inhibition was similar when con A was used at 0.3, 0.5, 1.0, 3.0 and 5.0 µg ml^{-1} but the same concentrations of dibutyryl cyclic AMP did not inhibit (and even enhanced) the response at con A concentrations ≥ 10 µg ml^{-1} (data not shown). Dibutyryl cyclic GMP (10^{-3} M) enhanced the con A-induced $^{45}Ca^{2+}$ uptake; when both reagents were added together (10^{-4} or 10^{-3} M of each) the inhibitory effect of dibutyryl cyclic AMP was overriding (Fig. 3). Neither cyclic nucleotide had a significant influence on the $^{45}Ca^{2+}$ uptake seen in cells incubated in MEM alone or with A23187. Cholera toxin (1.2×10^{-9} M), a potent activator of adenyl cyclase and theophylline (10^{-4} M), a phosphodiesterase inhibitor, both of which would be expected to increase intracellular cyclic AMP, also inhibited con A-induced $^{45}Ca^{2+}$ uptake, whereas 5′AMP (10^{-3}–10^{-5} M) or butyric acid (10^{-3}–10^{-5} M) were without effect (data not shown). Sodium azide (3×10^{-2} M), a respiratory inhibitor, had no influence on the uptake of $^{45}Ca^{2+}$ induced by con A added 30 min later (Fig. 3).

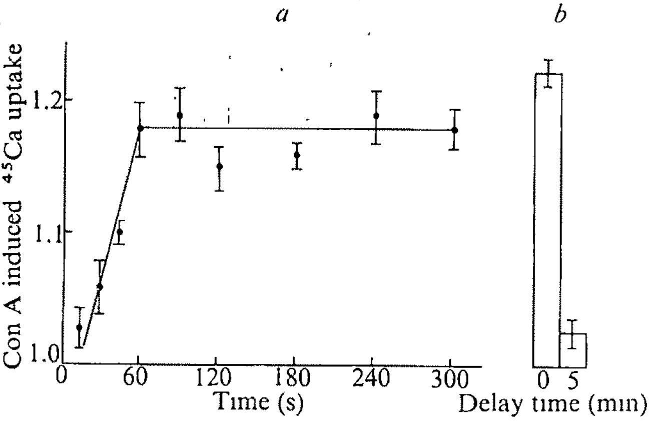

Fig. 2 *a*, Early time course of con A-induced $^{45}Ca^{2+}$ uptake in BALB/c spleen cells. Cells were incubated with 3 µg ml^{-1} of con A in MEM at 37 °C and sampled at times indicated. Uncorrected induced $^{45}Ca^{2+}$ uptakes were determined as in Table 1 and are expressed as means ±s.d. of triplicates. *b*, Results of two experiments where $^{45}Ca^{2+}$ was added, either at time 0, or 5 min after adding con A. In each case the time of incubation with $^{45}Ca^{2+}$ was 15 min. The results are expressed as means ± s.d. of of the means of the triplicates.

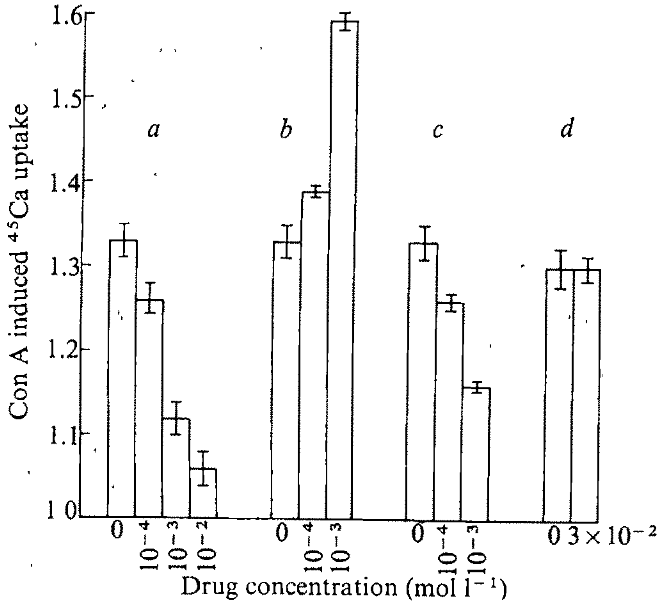

Fig. 3 Effect of *a*, dibutyryl cyclic AMP (Sigma); *b*, dibutyryl cyclic GMP (Sigma); *c*, dibutyryl cyclic AMP + dibutyryl cyclic GMP and *d*, sodium azide on uncorrected con A-induced $^{45}Ca^{2+}$ uptake. Cells were pretreated with drug or MEM at 30×10^{6} cells ml^{-1} for 30 min at 37 °C before exposure to isotopes and 3 µg ml^{-1} of con A for 15 min at 37 °C. The results of four separate experiments are expressed as means ± s.d. m. of each triplicate. In the absence of con A, these drugs did not significantly influence $^{45}Ca^{2+}$ uptake.

Are gated Ca^{2+} relevant for mitogenesis?

Our studies, which follow the pioneering work of Allwood *et al.*[6], and of Whitney and Sutherland[7,8], show that con A induces increased $^{45}Ca^{2+}$ uptake in mouse spleen cells, which is measurable by 45 s and complete by 1 min. Although soluble con A binds to all mouse spleen cells, only T lymphocytes are induced to divide and differentiate into lymphoblasts[13]. We have found that spleen cell populations depleted of T cells by treatment with anti-θ antiserum and complement, or derived from T-cell deficient *nu/nu* mice, do not show increments in $^{45}Ca^{2+}$ uptake on addition of con A. This proves that the increased uptake of $^{45}Ca^{2+}$ caused by con A is T-cell dependent, but does not identify the T cells unambiguously as being the cells displaying increased $^{45}Ca^{2+}$ uptake, though this seems most probable. It is unlikely that T cells are required to enable other cell types to take up $^{45}Ca^{2+}$, since the effect is (1) extremely rapid compared with T-cell secretory responses[23], (2) short and (3) not inhibited by high concentrations of sodium azide which would be expected to inhibit secretion by T cells.

It is interesting that mitogenic concentrations of bacterial LPS or pokeweed mitogen, both known to stimulate mouse B cell proliferation[13], did not induce measurable increments in $^{45}Ca^{2+}$ uptake in spleen cells from *nu/nu* mice, even though the LPS proliferative response has been shown to depend on extracellular Ca^{2+} (ref. 5). In normal spleen cell suspensions, pokeweed mitogen did induce increased $^{45}Ca^{2+}$ uptake, probably in T lymphocytes. This may reflect a difference in the mechanism of activation in T and B cells.

It is difficult to be certain that the con A-induced $^{45}\mathrm{Ca}^{2+}$ uptake truly reflects Ca^{2+} accumulation within the cell cytosol, and not increased adsorption at the cell surface. But the observations that the increased uptake is restricted to T cells (Table 3), that the effect is not seen if the cells are preincubated with con A for 5 min before the $^{45}\mathrm{Ca}^{2+}$ is added (Fig. 2*b*) and that it can be modulated by cyclic nucleotides (Fig. 3), all suggest that it is membrane transport, and not surface adsorption that is involved. Since, in general, extracellular Ca^{2+} concentrations are high ($\geq 10^{-3}$ M) compared with the concentration in the cytosol (10^{-5}–10^{-8} M)[24], the simplest interpretation of the increased $^{45}\mathrm{Ca}^{2+}$ uptake caused by con A is that it is related to the opening of 'Ca^{2+} channels' in the plasma membrane, which enables $^{45}\mathrm{Ca}^{2+}$ to enter the cell down a steep concentration gradient. One can calculate that the amounts of Ca^{2+} taken up by T cells stimulated by con A ($\sim 0.23 \times 10^{-9}$ M per 10^6 cells) would be large enough to raise the intracellular Ca^{2+} by 1.9 mM if all of the cell water ($\sim 122\ \mu\mathrm{M}^3$)[25] were available to the incoming Ca^{2+}. The rapid onset, brief duration and the resistance to inhibition by sodium azide of the induced $^{45}\mathrm{Ca}^{2+}$ uptake are consistent with this notion of gated Ca^{2+} channels.

It is interesting that rapid opening and closing of gated Ca^{2+} channels has been proposed to explain the phenomena of histamine secretion and desensitisation in rat peritoneal mast cells induced by the binding of antigen to IgE receptors in the presence and absence of Ca^{2+} respectively[26-28]. On the other hand, in their studies on PHA-induced $^{45}\mathrm{Ca}^{2+}$ uptake in human blood lymphocytes, Whitney and Sutherland found no evidence of gate closure: accelerated uptake of $^{45}\mathrm{Ca}^{2+}$ into PHA-treated cells continued for 24 h[7,8]. Whether this important discrepancy reflects differences in species, lymphocyte populations or methodology remains to be determined. We have found that con A, PHA and pokeweed mitogen behave similarly in this respect on mouse spleen cells.

The relationship between the lectin-induced $^{45}\mathrm{Ca}^{2+}$ uptake and DNA synthesis is far from clear. The findings that both responses occur only in T cells, show similar dose responses, and are inhibited by dibutyryl cyclic AMP and enhanced by dibutyryl cyclic GMP all suggest that they may be related. The following observations are also compatible with the idea that intracellular Ca^{2+} is a second messenger, which acts early in the sequence of events which lead to T lymphocyte proliferation and differentiation: (1) increased $^{45}\mathrm{Ca}^{2+}$ uptake is the earliest reported response and is not inhibited by ATP depletion caused by sodium azide; (2) all the early responses so far tested (enhanced turnover of phosphatidyl inositol[9] and increased amino acid transport[29]) and the late responses (proliferation and differentiation)[3-5] induced by con A or PHA have been found to be Ca^{2+} dependent; (3) pig[9] and human[10] lymphocytes can be induced to synthesise DNA and to differentiate into lymphoblasts by application of the Ca^{2+} ionophore A23187. On the other hand, the transient uptake of Ca^{2+} during the first minute after the binding of the lectin cannot be a sufficient irreversible signal leading to DNA synthesis, since removal con A from the cell surface (by addition of α-methyl mannosid at any time during the first 18 h aborts the proliferati response[30]. Since the removal of Ca^{2+} by addition of EGT during the first 12–36 h also prevents PHA-induced DN synthesis in human[4] and mouse[5] lymphocytes, it is clear th the requirement for extracellular Ca^{2+} is also a prolong one.

In addition to its possible relevance to lymphocyte prolifer tion, the ability to measure lectin-induced $^{45}\mathrm{Ca}^{2+}$ uptake simp and reproducibly in mouse T cells opens the way to studyi the properties of these putative 'gated channels' for Ca^{2+}. Su channels could be opened directly by con A binding to me brane glycoproteins which function as gating molecules, or some less direct mechanism. The finding that dibutyryl cyc GMP enhances, while dibutyryl cyclic AMP inhibits con induced $^{45}\mathrm{Ca}^{2+}$ uptake suggests that the operation of the gati event may be regulated by these cyclic nucleotides[31].

We thank Dr E. Simpson for supplying BALB/c and *nu/* mice, Dr M. Greaves for measuring the DNA synthesis in t experiment shown in Fig. 1, and Dr G. Janossy for supplyi some of the mitogens. M.H.F. is supported by a Visiti Scientist Award from the Canadian MRC.

No reprints of this paper will be available.

Received March 10; accepted April 14, 1975.

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letters to nature

Spectra of high-frequency radio emission from Jupiter

BETWEEN May 1972 and October 1974, observations were made from the University of Otago's field site at Swampy Summit near Dunedin, of Jupiter's high-frequency radio emissions. The frequency range of 23–25 MHz was reduced to a 2 MHz video signal, recorded on videotape, and analysed with a hi resolution spectrum analyser to produce the dynamic spectru of the millisecond radio bursts. The spectrum analyser w constructed from a modified video tape recorder, using the ti expansion principle developed independently by Ellis[1]. T frequency and time resolution were 10 KHz and 500 respectively, and the spectrum was recorded on 35 mm film the rate of 20, 40 or 100 ms per half frame.

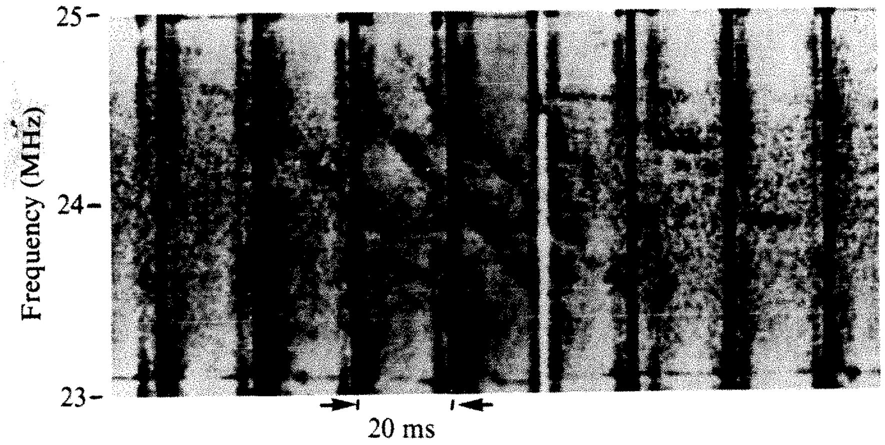
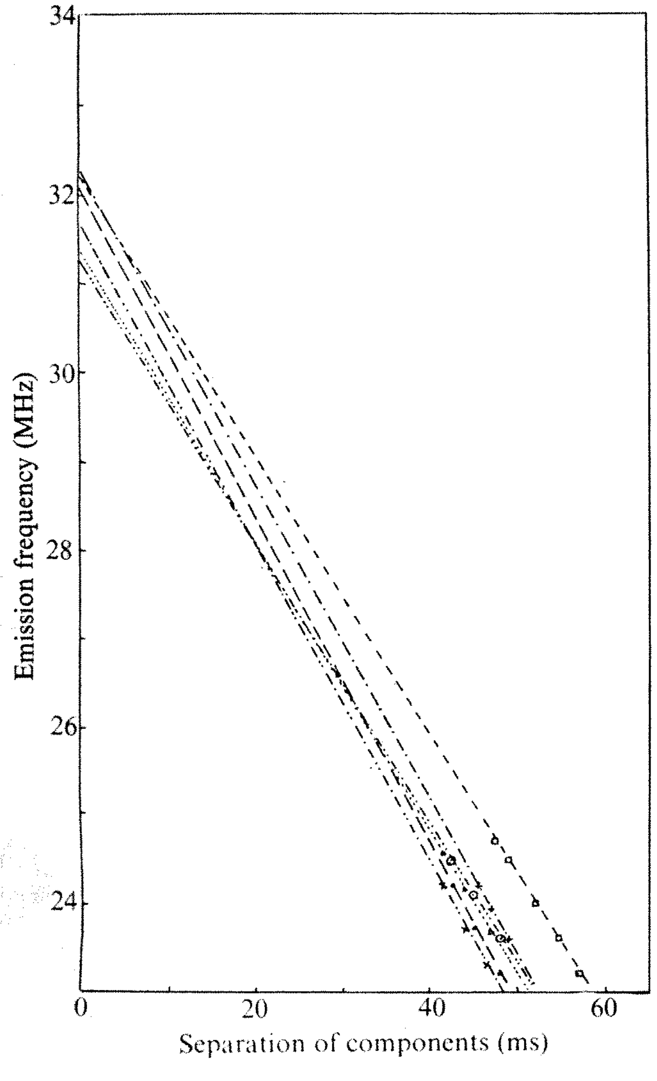


Fig. 1 The dynamic spectrum of Jupiter radio bursts: a closely-spaced doublet emission.

During the early source event of August 3, 1973, more than 75 min of videotape records were taken of the Jupiter radio bursts, producing over 1 km of film record showing more than 600 cases of millisecond burst spectra. Most of the emissions occurred in three burst groups lasting a total of less than 5 min, with the strongest burst group lasting for about 30 s.

A typical millisecond burst comprises a falling tone with a bandwidth of 20–150 KHz, and a frequency–time slope of −25 to −30 MHz s^{-1}. These values are in contrast with those of −10 to −15 MHz s^{-1} reported by Ellis[2], for observations at 15 to 17 MHz; they are, however, consistent with his predictions for cyclotron radiation from bunches of electrons moving along the spatially varying magnetic field of Jupiter.

Fig. 2 Separation of components in several widely-spaced millisecond bursts, as a function of frequency. The separations extrapolate back to zero in the region of 32 MHz.



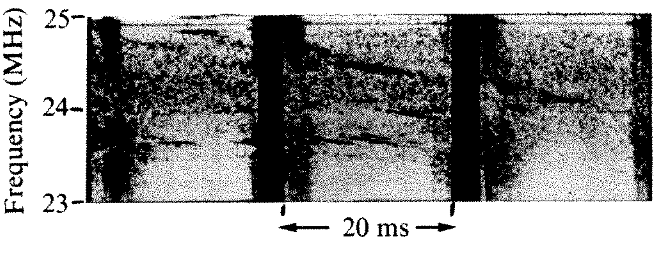
Multiple s-bursts which were proposed by some early observers but not confirmed, were common during periods of high emission rate. These occurred in pairs, and occasionally in triplets, with the component bursts each time having about the same frequency range, amplitude and slope. Separations between the components generally fell into the range 45–55 ms (widely spaced bursts), or 15–25 ms (closely spaced bursts), (Fig. 1).

A curious feature of the widely-spaced bursts is that the frequency–time traces of bursts are not quite parallel, but show consistent divergence, such that if the traces are extrapolated back in time they coincide at around 32 MHz. This extrapolation for several burst pairs and two triplets is shown in Fig. 2, where measurements of the component separation are plotted as a function of frequency over the observed range. The triplets are of particular interest, in that not only do the components extrapolate back to a common origin, but the middle component is also midway between the outer two at all frequencies (within the accuracy of measurement). It is difficult to determine whether the closely-spaced multiple bursts also show this divergence, as most of those bursts were too short for accurate measurement.

On a millisecond time scale, a considerable amount of fine structure was observed in the emission spectra (Fig. 3). Several discrete millisecond bursts were found to comprise shorter emissions with a time separation of 2–5 ms, and a frequency separation of 100–200 KHz. Other seemingly narrow-band bursts were actually made up of two or more very narrow band emissions (~20 KHz), with frequency separations down to 50 KHz. These emissions may be related to the fast lane bursts reported by Ellis, or could be a form of very closely spaced multiple bursts.

The properties of the multiple bursts fit Ellis's cyclotron model if we suppose that the components are emissions from closely spaced electron bunches moving in tandem along a

Fig. 3 High resolution dynamic spectrum of Jupiter s-bursts showing both a burst with overlapping structure and a burst with fine structure components with a frequency separation of ~70 kHz.



field line at about the same velocity as one another. The divergence with descending frequency of the temporal separation of the burst components and thus of the spatial separation of the electron bunches with distance up the field line, suggest that the bunches are formed at levels where the local cyclotron frequency is $\sim$32 MHz. The ordered spacing of the triplet components suggest that there is an ordered process of bunch formation, such as the breaking up of electron streams because of plasma instability. Only two strong triplets of adequate frequency range were, however, observed in this event. Clearly, more observations, preferably extending up to higher frequencies, would be helpful.

This work was supported by a grant from the New Zealand University Grants Council Research Committee.

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Received March 10; accepted April 9, 1975.

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Low frequency variability of BL Lac

The unusual object BL Lacertae has attracted attention because of its large and erratic variability at optical, millimetre and centimetre wavelengths[1-3]. The general form of the variations at short radio wavelengths is compatible[3-5] with the models of Van der Laan[6] and Ozernoy and Sazonov[7]. We report here observations of the variability of BL Lac at relatively long radio wavelengths which are in serious conflict with these models.

The observations were made at Jodrell Bank at λ74 cm (408 MHz) and λ31 cm (962 or 966 MHz) between July 1969 and December 1974. Extensive observations with the Jodrell–Mark III radio link interferometer show that BL Lac is completely unresolved at both wavelengths, with upper limits to its angular diameter of 2.0 arc s at λ74 cm and 1.0 arc s at λ31 cm. More limited observations with the Jodrell–Defford interferometer suggest that the angular diameter at λ74 cm is in fact smaller than 0.4 arc s. Because of this compact size, fringe amplitude measurements made with both short and long baseline interferometers at Jodrell Bank can be taken to provide measurements of the total flux density of BL Lac.

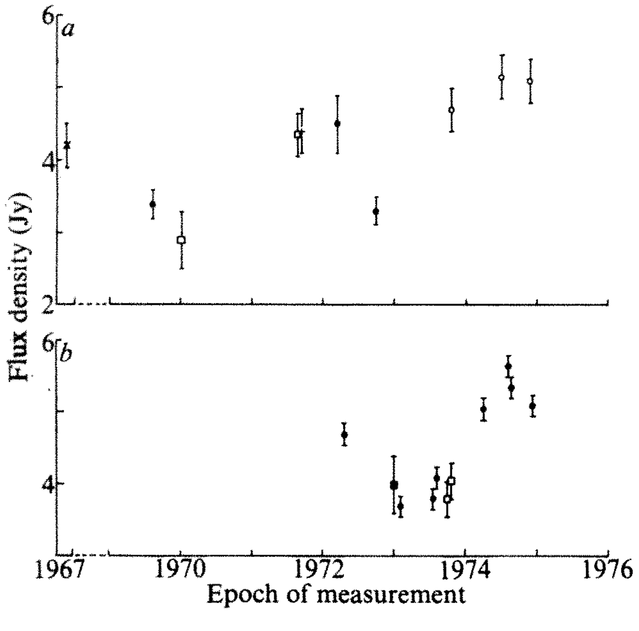

Fig. 1 The flux density of BL Lac at: *a*, λ74 and *b*, λ31 cm. ■, Measurements with the Mk IA single dish radiotelescope; ●, measurements with the Mk IA-II 425-m interferometer; □, measurements with the Jodrell-MkIII 23.8-km radio link interferometer; ○, measurements with the Jodrell-Defford 127.3-km radio link interferometer. + and ×, Measurements at λ74 cm made at Bologna and Cambridge, respectively. The significance of the error bars is discussed in the text.

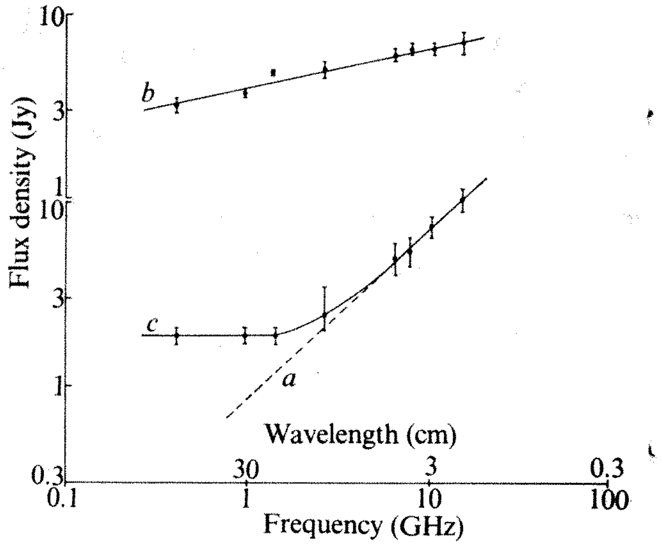

Fig. 2 The spectra of the quiescent and active components of BL Lac. *a*, Activity predicted at long wavelengths by the expanding cloud models; *b*, quiescent component; *c*, active component.

The measurements of flux density are shown as a function of epoch of observation in Fig. 1. The data obtained at λ74 cm are given on the flux density scale of Kellermann *et al.*[8] (KPW), with the assigned errors containing an allowance for the systematic uncertainties produced in converting the measurements made with different instruments (and often different calibration sources) to this common scale of flux density. Observations of non-variable sources with the same instruments suggest that the error estimates correspond to confidence limits at about the 90% level. The figure includes observations made at λ74 cm by Willson[9], and Fanti (personal communication). The data at λ31 cm were obtained primarily using the Mark IA–II interferometer, and have been calibrated on the nominal KPW scale at this wavelength by assuming the calibration source 3C295 to have a flux density of 29.8 Jy. The errors assigned to these measurements reflect only the amplitude repeatability of 3C295 and other calibration sources. For comparison with measurements made with other instruments and calibration sources, the errors shown on the Mark IA-II interferometer measurements at λ31 cm should be increased to ±0.25 Jy. The errors assigned to the λ31 cm measurements with the Jodrell–Mark III interferometer and the Mark IA single dish are the full formal errors on the KPW scale.

The data in Fig. 1 show that significant variations of about 2 Jy have occurred in the flux density of BL Lac at both λ74 and λ31 cm. Because of the uneven and often lengthy interval between the long wavelength flux density measurements we are unable to make a detailed comparison with individual events at short wavelengths. It is, however, possible to compare the general levels of activity using the procedure adopted by Ekers *et al.*[5]. Systematic monitoring at short wavelengths suggests that the activity of BL Lac can be well represented by a succession of discrete outbursts which are superimposed on some relatively stable 'quiescent' level. The physical significance of this base level is unclear; it may represent a steady non-varying component of the source, or it may merely be a complex level produced by the superposition of a number of decayed or small events. Whatever the cause, the apparent stability of the level does provide a useful reference, above which the activity can be defined.

We have used data from other variability studies[3-5,10-14], together with our own data, to assign values to the quiescent and active components of BL Lac between λ1.9 and λ74 cm. At each wavelength we have averaged the deepest minima in the intensity to define the base level of the quiescent component, and several of the highest peaks to obtain the general level of activity above this value. Because of the limited amount of data at λ74 and λ31 cm, the values we have assigned to the active component may well be an underestimate of the true activity at these wavelengths. The variability studies have, in general, differing time spans, so that it is necessary to assume that the level of activity at each wavelength did not change markedly between 1968 and 1974. The almost continuous runs of data by Medd *et al.*[10] and Andrew[4] suggest that this was the case at both λ2.8 and λ4.5 cm from 1968 until near the end of the summer of 1972. The variability seemed to cease for several months at this time, but we assume that this was only a temporary lapse, and that the activity at short wavelengths has since returned to its more usual level.

The spectra of the quiescent and active components of BL Lac are shown in Fig. 2. The quiescent component has an inverted spectrum with a frequency spectral index of approximately +0.2 (defined in the sense that $S \propto f^{\alpha}$). The variable component is flatter than this at low frequencies, but considerably steeper at high frequencies. As suggested by Ekers *et al.*[5], this marked difference in spectral characteristics supports the view that the quiescent spectral component may be associated with a discrete non-variable component of the source.

The activity of BL Lac at short wavelengths is in good general agreement[3-5] with the predictions of the expanding cloud model[6,15,16] and the application[7] of this model to ejected components. Although these models may not explain the detailed profiles of individual outbursts entirely satisfactorily[5], they do predict well the observed time delay between events at different wavelengths, and the inverted power law spectrum of the activity. The activity predicted by the models at long wavelengths is much less than that actually observed (Fig. 2*a*).

Not only do the conventional models for variability fail to account for the amount of activity at long wavelengths, but there is also a suggestion in the present λ31 cm data that the time scale of the activity may be somewhat different at these wavelengths. At short wavelengths BL Lac has an average of about 3 or 4 prominent outbursts each year. The activity at λ31 cm seems to have a considerably longer time scale of about 3 yr. Though it is true that our observations would not be particularly sensitive to variations on a time scale of a few months, there is little indication in the data of extensive short term activity of the type observed at short wavelengths. For instance, during a 30-d period in July and August 1973 we made almost daily observations of the flux density of BL Lac at λ31 cm. The measurements show only a gradual rise in intensity of about 0.3 Jy, with no evidence of short duration activity. At λ74 cm the observations are so infrequent that we can say little about the time scale of the variations. It is interesting to note, however, that the variations are superficially similar at both λ31 and λ74 cm, and can be fitted by a smooth curve which is in phase at the two wavelengths.

Hunstead[17] has reported variations in the λ74 cm flux density of the quasars 3C454.3 and CTA102, and the radio galaxies 1504–16.7 and 1524–13. The time scale of these variations is very similar to that of the variability reported here for BL Lac. Observations of the two quasars by Medd *et al.*[10] suggest that the activity at short wavelengths is apparently unconnected with the long wavelength variations. This is also true for BL Lac, and it is tempting to speculate that the same type of mechanism may be responsible for the low frequency activity in all five sources. The nature of this mechanism is as yet unknown.

We are grateful to colleagues for assistance with the observations, and thank R. G. Strom and P. N. Wilkinson for providing the early epoch measurements at λ74 cm. We thank R. Fanti and the Bologna group for permission to include their unpublished measurement A.M.T., R.W.P. and R.J.D. acknowledge financial support from the Science Research Council.

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Received March 10, accepted April 11, 1975.

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The output of the Etna volcano

ETNA shows two types of eruptive activity: persistent activity during which lava issues continuously at a low volumetric rate over a long time, and periodic eruptions during which lava issues at a high rate over a short time. The former is from vents at or near the summit, the latter most commonly from flank vents. In some flank eruptions the lava degasses from the central vent, and in others partly from the flank vents, building scoria cones there.

The historical record of activity on Etna is unrivalled in the large number of lava flows of known date and areal extent: Sartorius von Waltershausen[1] compiled a map of the lavas formed before 1839, and each eruption since then has been documented. Nevertheless, few attempts have been made to determine the volumetric output of new rock over the historic period, in spite of the importance of such data to understanding the mechanism of the volcano.

The problem of determining the output is not, however, straightforward, because although the volume of each dated, flank lava flow can be readily estimated, it is difficult to assess the contribution from persistent summit activity. We consider here the present output and attempt to relate this to the earlier history.

The years immediately before 1971 were characterised by persistent activity from the north-eastern crater. During that time we made estimates of the effusion rate on three occasions (refs 2 and 3, and G. P. L. W., unpublished) and arrived at a consistent value of around 1 $m^3 s^{-1}$. The accumulated products of the persistent summit activity from 1932 to 1967 amount to 270×10^6 m^3, based on a comparison of two editions of the 1:25,000 topographic map of Etna; flank activity during this period contributed a further 60×10^6 m^3, giving a total of 330×10^6 m^3 or 0.3 $m^3 s^{-1}$ averaged over this period of 35 yr.

The persistent activity ceased with the commencement of an eruption during the spring of 1971 but resumed again in late

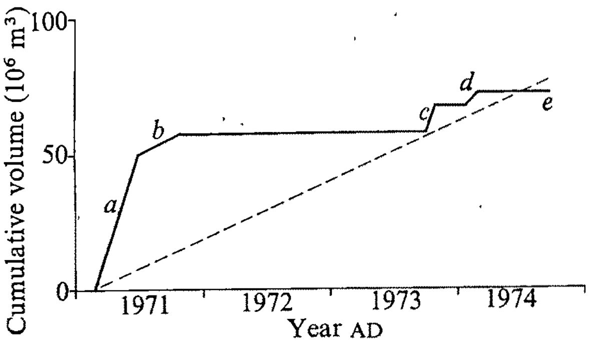

Fig. 1 Cumulative output of Etna by summit and flank activity during the period April 1971–September 1974. *a*, 1971 eruption; *b*, partial infilling of the Chasm; *c*, partial infilling of the Chasm and Bocca Nuova; *d*, January–March 1974 flank eruption; *e*, resumption of persistent activity from the North-eastern Crater. Dashed line, average output rate (0.68 $m^3 s^{-1}$).

1974. Between the 1971 eruption and late 1974 there were, however, several eruptive events, notably a partial infilling of the Chasm (the main crater at the summit of Etna) by lava in 1973. A small flank eruption also occurred in January–March 1974 (ref. 4). The estimated volume of lava produced during all the activity from spring 1971 to the present (Fig. 1) gives an average rate of 0.68 $m^3 s^{-1}$. These figures compare with the higher average rate of 3.4 $m^3 s^{-1}$ for Kilauea[5].

Reasonably complete records of flank eruptions span the period from 1535 to the present. One of us[6] has measured the area of each historic flank flow and has visited each locality to estimate the average thickness of the flows. The assessed volumes (Fig. 2) are, in general, smaller than volumes given in the literature, but if there is any subjective error in estimating the thickness of the flows it should be consistent.

The striking feature (Fig. 2) is that there were four periods of contrasted outputs: first, 1535–1610, characterised by a rather low effusion rate; second, 1610–1669, characterised by a high effusion rate, averaging 0.83 $m^3 s^{-1}$; third, 1669–1763, during which there were no major flank eruptions; fourth, from the 1759 resumption of flank activity to the present, characterised by a uniform and moderate effusion rate averaging 0.17 $m^3 s^{-1}$.

The high effusion rate between 1610 and 1669 could be correlated with the absence of summit activity during that period: flank eruptions accounted for the entire output. Following that period a large caldera formed at the summit during the 1669 flank eruption, and the contemporaneous absence of flank eruptions could have been because the steady volcanic activity at the summit was filling in the caldera. By the time of Spallanzane's visit at the end of the eighteenth century[7], Etna again had a summit cone standing above the caldera rim.

Persistent summit activity occurred more or less continuously between 1759 and the present, and the average effusion rate of 0.17 $m^3 s^{-1}$ resulting from the flank activity is thus only part of the total. It is impossible to arrive at an accurate figure for the contribution by persistent summit activity. In particular, there are many undated lava flows which originated high on the volcano belonging to that period. A rough calculation of the volume of Etna standing above the 1669 caldera rim, and the volume of known summit lava flows of the past 200 yr gives a total of $500 \times 10^6 m^3$, and an average effusion rate of 0.09 $m^3 s^{-1}$. Some pyroclastics have fallen beyond the caldera rim or have been eroded from the summit cone, but a total output of close to 0.26 $m^3 s^{-1}$ for the period is indicated.

A volcano such as Etna is a system into which magma rises from the mantle and out of which lava is erupted at the surface. The input is generally greater than the output since some, perhaps rather more than 10% of the magma which enters the volcano congeals below the surface to form intrusions. There is general evidence that the input is continuous, and that high level magma reservoirs exist, within which some of the input is stored between eruptions.

A model which can reconcile the varied output over the past 450 yr with a uniform input rate is one in which there are two magma reservoirs within or below Etna filling and emptying independently of one another. The smaller reservoir would have a capacity of the order of $50 \times 10^6 m^3$ and would be partially or totally drained during normal flank eruptions such as have occurred roughly every 10 years between 1763 and the present. The large reservoir would be of an order of magnitude larger and would probably take hundreds of years to fill: the last time it emptied was in the seventeenth century. The most cogent evidence for the existence of this larger reservoir is the caldera collapse which took place at the summit of Etna during the 1669 flank eruption and terminated the period of high output (Fig. 2).

On this model, the input rate is constant at perhaps 0.3–0.4 $m^3 s^{-1}$. The output during the period 1610–1669 was greater because of the release of the contents of the larger reservoir which had filled over a long period. The output during the two later periods was smaller because slow refilling of the large reservoir then absorbed part of the input.

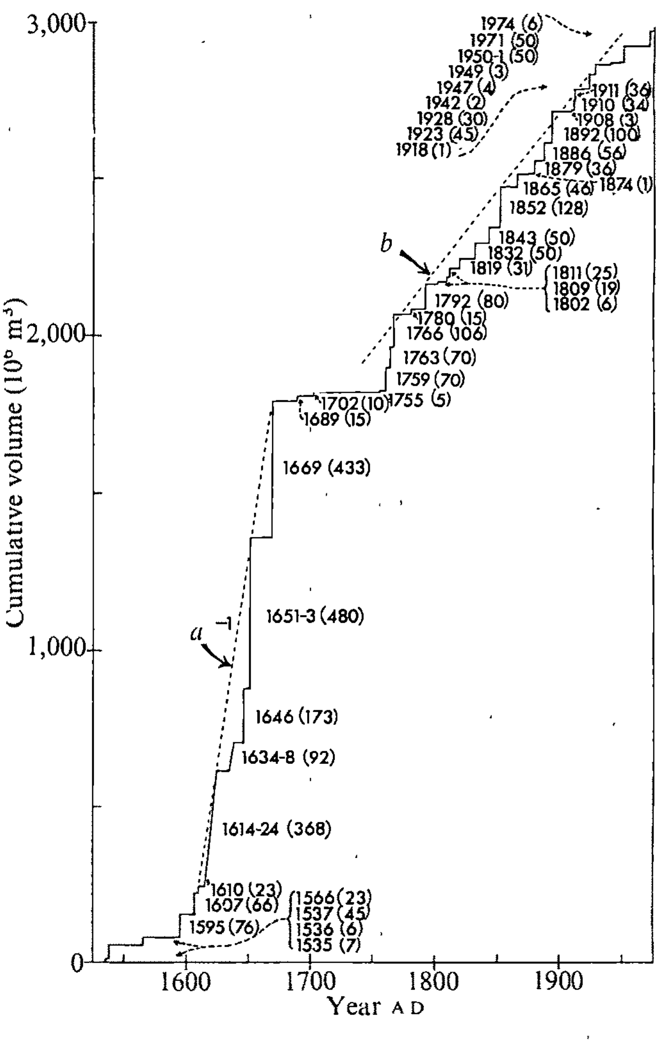

Fig. 2 Cumulative output of Etna by flank activity since 1535. Some of the eruptions listed in ref. 9 are omitted (1540, 1550, 1578–80—small eruptions, no volumetric data available; 1883—no lava flow produced; 1682, 1747, 1869—summit eruptions; 1764–45—confusion exists regarding the source, but very probably persistent activity; 1603—dubious eruption), but probably do not change the picture significantly. *a*, Average output rate: 0.83 $m^3 s^{-1}$; *b*, average output rate: 0.17 $m^3 s^{-1}$. Numbers in brackets following dates give the volume of output ($10^6 m^3$) for those dates.

A second model which can reconcile a varied output with a uniform input requires only one storage reservoir, and the nature of the eruptive activity depends on the state and, particularly, the height of the volcano. Persistent activity is a steady leakage which acts as a form of safety valve. Perhaps it can release the entire input, although it normally releases only a part and flank eruptions release the remainder. Persistent summit activity steadily increases the height of the volcano, and because the hydrostatic pressure in magma at the summit decreases as its height increases[8] this results in a reduced leakage rate. A greater proportion of the input is stored, and the resulting inflation of the volcano then increases the likelihood of major flank outbreaks, as in the seventeenth century. The major channelways so opened divert summit activity to the flanks. The loss of a large volume of magma from the storage reservoir results in caldera collapse, thus terminating a cycle. One possible objection to this model is that the height of Etna has not increased significantly in the past century (1864—3,313 m; 1900—3,274 m; 1973—3,316 m). Also, a gradual decrease in leakage rate could be expected to lead to a gradual increase in the magnitude of flank eruptions, rather than an abrupt increase as occurred in the seventeenth century.

Both of these models envisage a uniform rate of input, but another possibility is that the input itself varies with time and was particularly high during the period of high output in the seventeenth century. The eruptions of this period include two (those of 1614–24 and 1651–53) which produced pahoehoe instead of the more normal aa flows of Etna, and these flows probably had an unusually low viscosity. If the low viscosity resulted from a difference in chemical composition then that, and the increased output, could well correlate with changed conditions in the mantle. The large volume of lava which emerged in the 1669 eruption, however, can be related to the unusually low level of the 1669 vents[8].

There is at present no way to choose between these three alternative explanations for the variation in output. Whichever applies, we see signs of a cyclic pattern, each cycle lasting for some hundreds of years and terminated by caldera collapse. There are traces of two calderas (the Valle del Leone and Cratere Ellittico) which existed earlier than that of 1669 (the Cratere del Piano), and the Valle del Bove may be another. There are also lava flows of large volumes, which are older than the seventeenth century.

The discrepancy between the low output (0.26 m^3 s^{-1}) of the past 200 yr and the higher output of the most recent period (0.68 m^3 s^{-1}) could be attributable to several factors. One possibility is that the latter indicates the onset of another highly voluminous effusive period such as that of the seventeenth century, although there is no other supporting evidence for this.

The base level of Etna varies from below sea level to more than 1,000 m OD. The volume of Etna standing above a horizontal plane 500 m OD is 515 km^3. To this should be added 12 km^3: the volume deficit of the Valle del Bove. The total output since 1535 is of the order of 3.6 km^3, or an average of 0.26 m^3 s^{-1}. At this rate Etna could have been constructed in 65,000 yr.

Understanding the mechanism of Etna depends on the availability of good quantitative data. This study demonstrates the importance of making more critical and more frequent measurements of the output.

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Received March 18, accepted April 15, 1975.

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Large sudden warming in the Southern Hemisphere

LARGE and rapid temperature changes, 'sudden warmings', can occur in the stratosphere during winter[1]. They are hemispheric-scale phenomena, and, until recently, were supposed to be much weaker in the Southern Hemisphere winter than in the Northern Hemisphere winter. But during July 1974 a warming occurred in the Southern Hemisphere which was more intense in the initial phase than any of the three very large warmings of the past four Northern Hemisphere winters. The relatively early date of this warming (most large warmings in the Southern Hemisphere occur after late August at the end of the winter), its failure to produce the large heating of the polar cap which normally occurs in the Northern Hemisphere after such intense initial activity, and the large associated cooling in the tropics and Northern Hemisphere are further reasons for regarding this as a significant event.

Measurements by the Selective Chopper Radiometer (SCR)[2–5] carried by Nimbus 5, give continuous coverage of atmospheric temperature between 80°S and 80°N. Uninterrupted measurements of a layer approximately 20 km thick and centred near 150 Pa (1.5 mbar) (~45 km near the stratopause) have been made since April 1970 using this instrument or its predecessor on Nimbus 4. Both instruments operate by measuring thermal emission from atmospheric carbon dioxide in the 15-μm band in the infrared, using cells of carbon dioxide acting as filters in series with interference filters. The satellites are in Sun-synchronous polar orbits, passing over most places near local noon and midnight; the orbits are 26.8° longitude apart.

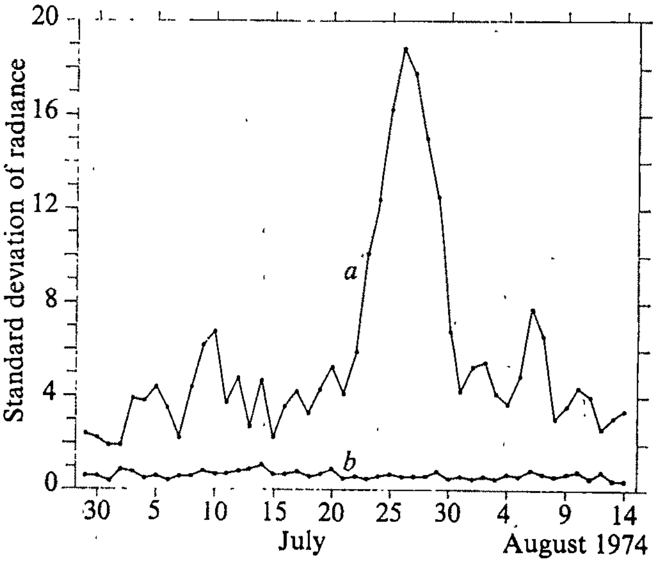


Fig. 1 Standard deviation of channel B23 radiance (mW m^{-2} sr^{-1} (cm^{-1})$^{-1}$) around latitude circles. This channel measures emission from the upper-stratosphere. *a*, 60°S; *b*, Equator.

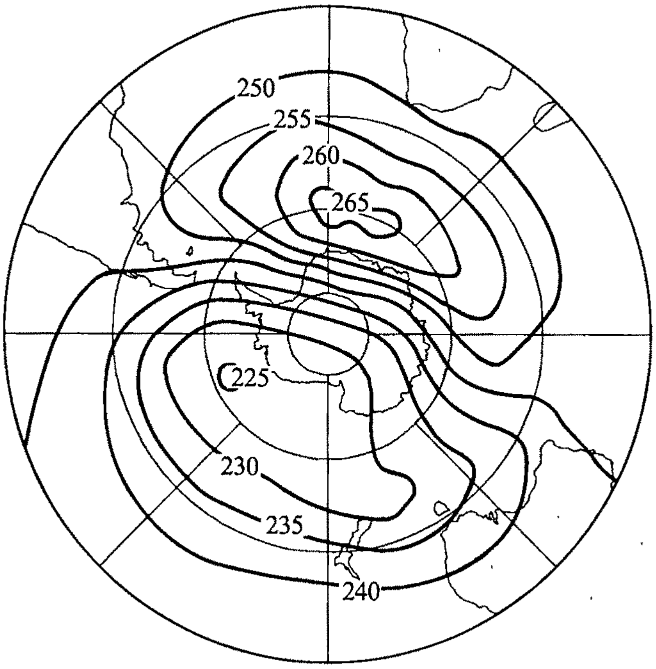


Fig. 2 Channel B23 equivalent temperature (K) on July 26, 1974, on a polar stereographic projection of the Southern Hemisphere bounded at 20°S.

A measure of the eddy activity (deviation from zonal symmetry) in the winter stratosphere is given by the temperature variation around a latitude circle at a middle or high latitude. Variations are normally largest at 60–65° latitude as in this case, and Fig. 1 gives standard deviations for channel B23 at 60°S. The weighting function of this channel has a peak near 400 Pa (~33 km); most of the measured emission originates in the 1,600–140 Pa layer (~28–46 km). The radiance unit is mW m^{-2} sr^{-1} (cm^{-1})$^{-1}$, and for points in this figure an interval of one radiance unit is equivalent to about 1 K temperature change. A large peak of 18.8 radiance units was centred on July 26, 1974; for nine days standard deviations were larger than the norm of about five units for that winter. Figure 2 gives an analysis for this day and shows that the pattern was almost entirely wavenumber one, with a hot centre at 60°S 10°E and a cold centre at 64°S 120°W. Table 1 gives the maximum standard deviations around latitude circles for the largest warmings observed since April 1970. Not only was the warming of July 1974 the largest Southern Hemisphere warming, but it was the earliest, and was weaker than only the two Northern Hemisphere warmings of 1971 and that of December 1974.

Table 1 Warmings between April 1970 and February 1975 during which standard deviation of radiance around a latitude circle exceeded 12 radiance units

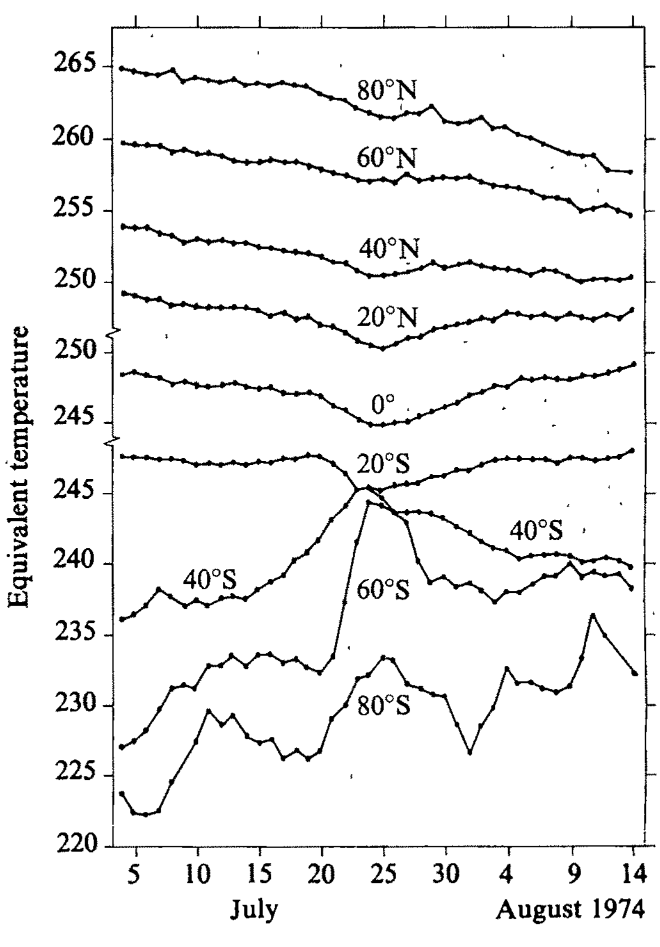
Date	Channel	Latitude	Standard deviation
Nimbus 4			
August 5, 1970	A	48°S	14.8
September 5, 1970	A	60°S	15.4
September 23, 1970	A	60°S	16.6
October 5, 1970	A	60°S	13.2
October 18, 1970	A	68°S	16.3
January 3, 1971	A	64°N	31.3
March 14, 1971	A	72°N	22.0
September 7, 1971	A	60°S	14.7
January 10, 1972	B	68°N	14.1
February 13, 1972	A	60°N	17.7
March 26, 1972	A	64°N	17.7
July 29, 1972	A	64°S	14.4
Nimbus 5			
January 28, 1973	B23	68°N	18.0
August 16, 1973	B23	64°S	12.7
February 25, 1974	B23	68°N	13.3
March 16, 1974	B12	64°N	13.3
July 26, 1974	B23	60°S	18.8
December 30, 1974	B23	68°N	21.2

Large wave activity is normally accompanied by a rapid temperature rise at the pole because heat is transported poleward by the eddies at an increased rate. Figure 3 shows a temperature maximum on July 25 at 80°S, temperature having increased by 7 K during the previous 6 d. But the Northern Hemisphere warmings of January 1971, January 1973, February 1974 and December 1974 produced rises of zonal mean temperature at 80°N of about 33 K, 25 K, 35 K and 36 K respectively at this level, to give higher temperatures than at the Equator, although all except the first and last were associated with smaller eddy maximum standard deviations than that of July 1974.

Figure 3 shows that the rise of zonal mean temperature at 60°S was larger than at 80°S, although still small by Northern Hemisphere standards. A better measure of the height of these peaks of zonal mean temperature is given by the average of the rise before the peak and the fall afterwards. This was still largest at 60°S, 8.9 K compared with 7.0 K at 80°S. The maximum at these latitudes was accompanied by a minimum between 20°S and 40°N (Fig. 3), this being most marked at the Equator where temperature was 2.7 K lower on July 25 than the average of July 19 and August 1. Such tropical and summer hemisphere cooling is well known to occur with large Northern Hemisphere warmings[7,8] and has been demonstrated for a Southern Hemisphere warming[7], but this is the largest cooling reported (or observed by the SCRs) for a Southern Hemisphere warming.

The pattern of events differed from that which is normal in

Fig. 3 Temperature (K) equivalent to the zonal mean radiance measured by channel B23 (upper stratosphere). Values are given each day.



the Northern Hemisphere. There the maximum of zonal mean temperature is normally most pronounced at the pole and is about eight times as large as the low latitude and Southern Hemisphere cooling, which occurs simultaneously; the large eddy activity normally precedes the maximum by at least a day. Here the maximum eddy amplitude coincided with the extremes of zonal mean temperature, although the equatorial cooling was 30% of the maximum temperature rise, which occurred not at the pole but at 60°S. Similar but smaller changes in the zonal mean were shown by Fritz and Soules[7] for a lower level in the Southern Hemisphere.

Why did the large amplitude waves during July 1974 not lead to large polar heating? Data from other levels show that the temperature anomalies tilted towards the west with increasing height and decreasing distance from the Equator. These tilts are instrumental in producing heat and momentum fluxes[8], the relative and absolute magnitudes of which must determine the behaviour and size of the warming. But the tilts were about half as steep as has been typical in a Northern Hemisphere warming, and the wave amplitudes in the lower stratosphere were also very much smaller. Both these factors imply reduced eddy fluxes, so perhaps the pertinent question is why the equatorial cooling was so large when changes of average temperature at high latitudes were not so extreme. These changes had no significant effect on the global mean radiance at any level, so the warming merely redistributed the heat horizontally, as would result from horizontal eddy heat flux or adiabatic heating as a result of meridional circulations[10].

I thank Dr J. T. Houghton and members of this department for assistance, and the SRC and the NERC for financial support. NASA provided technical support.

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Received April 7; accepted April 23, 1975.

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Sources of magnetic anomalies on the Mid-Atlantic Ridge

STRONGLY magnetised basalts occur at the basement surface in ridge crest areas (see, for example, refs 1, 2) and various models for the magnetic sources have been devised. A uniformly magnetised layer, extending downwards from the basement surface for 400–500 m is consistent with most of the geophysical information, although some older estimates and the low magnetisation of some sets of rocks[3] require a layer thickness of as much as 2.5 km. During Leg 37 of the Deep Sea Drilling Project[4] five basement holes, all deeper than 100 m, were drilled from DV Glomar Challenger along a seafloor spreading flow line from the median valley of the Mid-Atlantic Ridge in the project FAMOUS survey area into the American Plate (Fig. 1). Basement penetration ranged from 108 m to 582 m and totalled 1,453 m with 243 m recovery. Here we report the results of a shipboard palaeomagnetic reconnaissance of 295 vertically oriented basement samples.

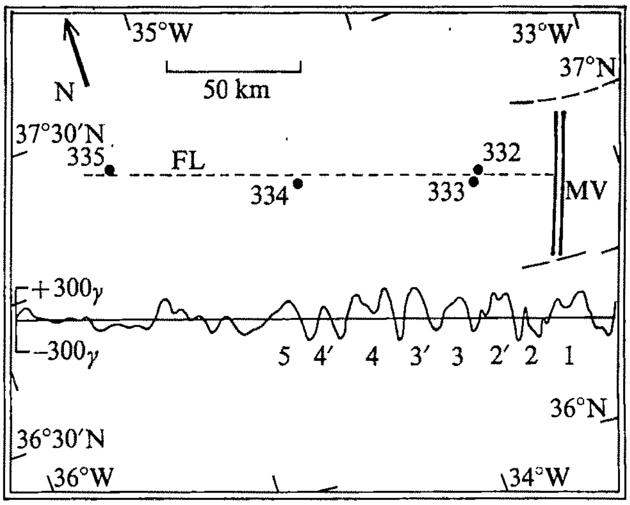
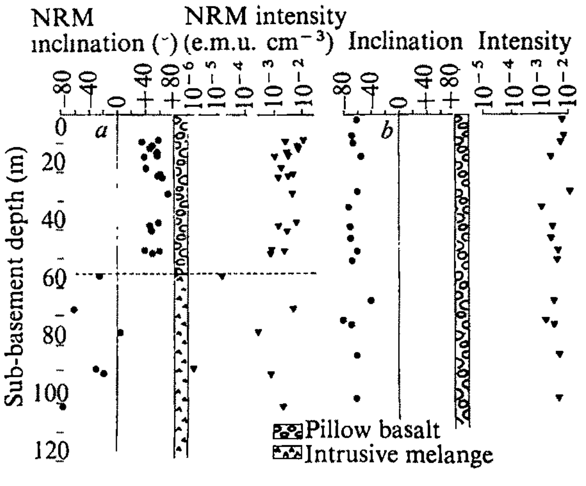


Fig. 1 Location of DSDP Leg 37 sites along a seafloor spreading flow line (FL) to the west of the Median Valley (MV) of the Mid-Atlantic Ridge. The magnetic anomaly profile along the flow line is shown with the anomalies numbered following Talwani *et al.*[5]

At the two oldest sites, 334 at 9 Myr and 335 at 11–16 Myr, the uppermost basement sections consist of monotonous rather massive pillow basalts which are strongly magnetised with sharply defined inclination ranges (Fig. 2). At site 334, located within positive magnetic anomaly 5, 55 m of normally magnetised basalts (mean inclination (± s.d.m.) for 18 samples, $\bar{I}$, of 54° ± 2°) overlie a mélange of gabbros and serpentinised peridotites with scattered but generally upward magnetic inclinations. Here and at site 335 (108 m of basalt penetrated, with 15 samples yielding $\bar{I} = 65 \pm 1°$) basalt magnetic polarity matches the sense of the magnetic anomaly at the site.

But at sites 332 and 333, both with an estimated basement age of 3.5 Myr, and located within the negative anomaly between 2′ and 3′ corresponding to part of the Gilbert reverse polarity epoch, the basement lithological and magnetic sequences (Fig. 3) are complex with rapid vertical and lateral variation. Extrusive and possibly intrusive types of basalt occur, together, in the uppermost 300 m of basement, with very extensive zones which, because they drill very rapidly with negligible recovery, are interpreted as consisting of basalt rubble and sediment. In the 333-m thick basement section of hole 332A an 80-m thick normal (N) polarity zone overlies a 130 m thick dominantly reverse (R) polarity zone. The lowest 120 m of this hole, represented by 35 samples from at least 10 cooling units, is dominated by magnetic inclinations close to the horizontal. These shallow ($+20 \geq I \geq -20°$) anomalous inclinations are found extensively in all three deep basement holes in this area (332A, 332B and 333A). Hole 332B, situated only 100 m laterally from 332A, and penetrating basement to a

Fig. 2 NRM inclination and intensity profiles based on individual sample values for: *a*, site 334; *b*, site 335.



depth of 582 m, has a markedly different inclination profile. The 80-m thick N polarity zone at the top of the basement section in hole 332A is missing. Although visually similar, distinctive coarse grained plagioclase–phyric basalts cap the basement sections in both holes, and the polarities are opposite being N in 332A and R in 332B. The differences between the 332A and 332B basement sections are surpassed by those between 332B, 332C and 332D, the last two only sampling the uppermost basement unit during attempts to re-enter hole 332B. All three holes are located within 10 m of each other and the topmost basement unit at 332C is the same reversely magnetised, coarsely plagioclase–phyric basalt as at 332B, but the topmost unit at 332D is a strongly normally magnetised aphyric basalt.

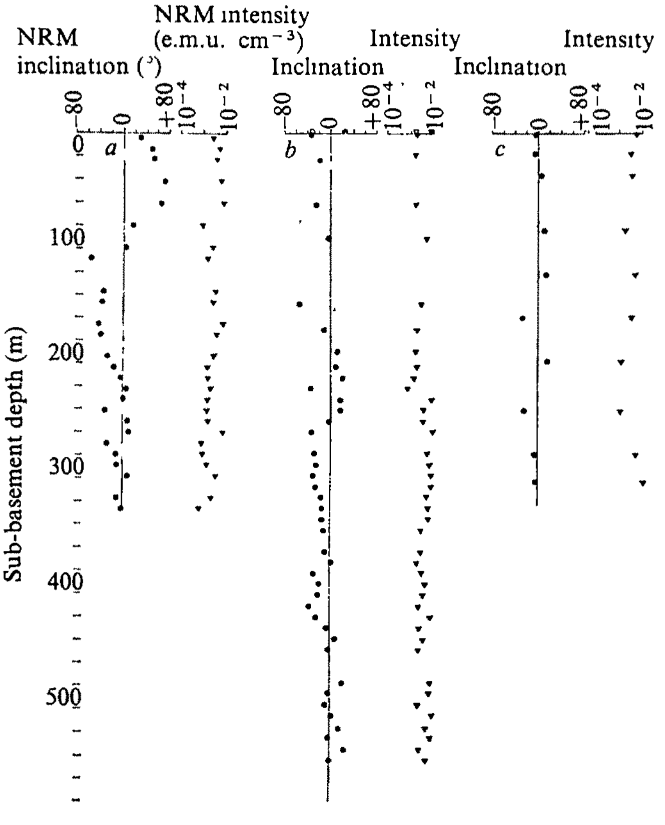
Any model for basement formation at site 332 requires accumulation during at least two geomagnetic polarity epochs of opposite senses. These may be contiguous epochs if the sequence consists of superimposed lavas, or widely separated if intrusives are present.

An important result for anomaly generation is the absence of any overall trend with depth in the magnetic intensity of the basalts (Figs 2 and 3). Magnetic intensity does change rapidly over small vertical intervals, but this can be attributed to petrological variation and degree of alteration. Thus, no direct evidence is available on the question of the nature of the lower limit of a magnetic source layer.

Modelling, using the representative values of magnetic intensity now available for each site, gives an alternative method of estimating magnetic source thickness.

At the two older sites, 334 and 335, the average basalt magnetic intensity, giving unit weight to mean values for each 9.5 m core length, is close to 40×10^{-4} e.m.u. cm^{-3}, or 32×10^{-4} e.m.u. cm^{-3}, if allowance is made for the presence of effectively non-magnetic randomly oriented basalt rubble and essentially non-magnetic sediments. With these intensities and the observed steep inclinations, a magnetic source layer extending downwards from the basement surface for from 570 m to 820 m will suffice to produce the observed anomalies. This range in thickness extends from values comparable with previous estimates to about a factor of two more.

Fig. 3 NRM inclination and intensity profiles based on 9.5 m core length average values for: *a*, site 332A; *b*, site 332B; *c*, site 333.



Although these estimates of the thicknesses of magnetic anomaly source layers are based on more representative information than all previous estimates, they still cannot be taken as generally applicable, as is clear from the presence of only 55 m of basalt at site 334 and the complex lithological and magnetic situations at sites 332 and 333. The lack of a thick basaltic magnetic source layer at site 334 is surprising since the site is well within a clear example of magnetic anomaly 5, an anomaly that, after the central positive anomaly 1, is usually the easiest to recognise on all spreading ridge systems.

At sites 332 and 333 the main magnetic sources required to produce the anomaly pattern are not present in the sections drilled, which extend to 582 m below the basement surface. Although occasional highly magnetic basalt samples (up to 175×10^{-4} e.m.u. cm^{-3}) were found throughout the basement sections, the combination of the presence of abundant basalt rubble and sediment, together with commonplace shallow inclinations and polarity reversals (Fig. 3) reduces the effective magnetisation of these sections to an insignificant level. Only a 57-m thick sequence of massive picritic pillow basalt in hole 332B, physically akin to the sites 334 and 335 basalts, and with its top at close to 270 m sub-basement, is potential anomaly source material. The average magnetic properties, giving unit weight to mean values for each 9.5 m core length, for the basement sections of holes 332A, 332B and 333A are intensity 39×10^{-4} e.m.u. cm^{-3} (or 21×10^{-4} e.m.u. cm^{-3} if allowance is made for non-magnetic material) and inclination −04°. A magnetic anomaly source layer with these properties would have to be in excess of 2.5 km in thickness to produce the observed anomalies. This figure exceeds the local thickness of volcanic layer 2. Modelling using an inclination of −04° for the reversely magnetised block in which sites 332 and 333 are located, and ±60° elsewhere (implying that the reversed anomaly is effectively produced by a gap in a normally magnetised layer), with a source layer thickness of 570 m throughout, produces a poor fit with observation in the vicinity of sites 332 and 333. To obtain a satisfactory fit it is necessary to assume either that the shallow inclination magnetisation of the top 600 m of layer 2 has an average azimuth well away from the present magnetic north–south direction, or that the magnetic source layer is confined to depths in excess of 600 m sub-basement. Lack of trends with depth in basalt magnetic intensity allow for the latter possibility.

We thank Captain J. C. Clarke, the crew, drilling and laboratory teams on Glomar Challenger for help. Patrick Ryall of Dalhousie University computed magnetic source layer thicknesses using programs supplied by David Ross and S. P. Srivastava. Dalhousie University and the National Research Council of Canada equipped the shipboard palaeomagnetic laboratory. The Deep Sea Drilling Project is funded by the National Science Foundation and managed by the Scripps Institution of Oceanography.

SCIENTIFIC PARTY
DEEP SEA DRILLING PROJECT LEG, 37*

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Received November 19, 1974, accepted April 1, 1975.

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Geological interpretation of whole-rock isochron dates from high grade gneiss terrains

Many Rb–Sr whole-rock isochron dates have been reported on extensive terrains of quartzo-feldspathic orthogneisses. Most are of Precambrian age. In most cases the date is either regarded simply as the "age" of the gneiss complex (with no specific interpretation), or as the age of regional metamorphism. The latter interpretation naturally implies large scale regional homogenisation of Sr isotopes at the isochron date. I find such an interpretation improbable.

First, good "primary" Rb–Sr whole-rock isochrons are commonly observed in gneiss terrains which underwent much later intense metamorphism (at least up to amphibolite facies) indicating isochemical behaviour with respect to Rb and Sr on a whole-rock scale. Thus, Scourian gneisses of north-west Scotland yield Rb–Sr whole-rock dates of about 2,700 Myr, in spite of being subjected to intense Laxfordian metamorphism and deformation nearly 1,000 Myr later, as shown by mineral dates[1,2]. Similarly, the Amîtsoq gneisses of West Greenland have preserved their 3,700 Myr Rb–Sr whole-rock isochron systematics in spite of amphibolite facies metamorphism and intense deformation at about 2,600–2,800 Myr ago[3,4]. Many analogous cases have been reported.

Second, Krogh and Davis[5] have demonstrated unequivocally from Rb–Sr laboratory studies on gneisses of the French River area of the Grenville Province of Ontario that isotopic and chemical equilibrium was not reached over distances of even a few centimetres during a regional amphibolite facies metamorphism which occurred some 800 Myr after their "formation".

Why, then, do extensive gneiss terrains frequently yield such good Rb–Sr whole-rock isochrons, and what do these isochrons really mean? I have suggested that low, upper mantle type, initial ^{87}Sr/^{86}Sr ratios of many ancient gneisses indicate that their immediate precursors are predominantly juvenile additions to the continental crust at, or close to, the measured age[6]. The interval between times of extraction of juvenile igneous material from the upper mantle and/or subducted basic lithosphere and time of production of the regional metamorphic characteristics of the derived gneiss complex may be less than 50–100 Myr, which falls within the analytical uncertainty of most age measurements on Precambrian rocks. In my model major crustal accretion is regarded[6] as essentially contemporaneous with the profound geochemical and petrological differentiation required to yield a compositionally gradational crust, in which the raw materials of granite (*sensu lato*), as well as geochemically incompatible elements and water, migrate upwards, leaving behind depleted granulite facies rocks at depth[7,8].

On such a model, Rb–Sr whole-rock isochrons on orthogneisses simply give the age of the accretion–differentiation event. The best isochrons are obtained when the duration of the latter event is short in relation to its actual age. A low, homogeneous, initial ^{87}Sr/^{86}Sr ratio is inherited directly from the upper mantle or basic lithosphere source region, while local and regional dispersion in Rb/Sr ratios within the gneiss complex results from synchronous geochemical differentiation processes. Significant deviations from ideal Rb–Sr isochron behaviour are quite common, and may be attributed to source region heterogeneities, to interactions of the gneiss precursors with pre-existing crust, or to lack of closed system behaviour on a whole-rock scale with respect to Rb and/or Sr during subsequent metamorphism and tectonism.

These considerations also apply to Pb–Pb whole-rock isochron dates on ancient orthogneisses, which are frequently in good agreement with corresponding Rb–Sr dates [2,9,10]. In such cases, simplicity of the Pb–Pb isotope systematics suggests derivation from a homogeneous U–Pb (usually expressed as ^{238}U/^{204}Pb) source region approximating to single stage evolution from time of formation of the Earth to the measured isochron date[11], although small deviations from ideal behaviour are common. Consideration of the actual ^{238}U/^{204}Pb value of the source region (about 7.3–8.0, using the model parameters of Oversby[12]) as calculated from the primary Pb isotope growth curve and of the customarily close agreement of a given Pb–Pb isochron date with that obtained from the intersection of the Pb–Pb isochron with the primary growth curve, suggests that the source region of the gneiss precursors was upper mantle or basic lithosphere.

Synchronous geochemical differentiation of newly accreted sial produces severe U–Pb fractionation at the measured isochron date[13]. The wide range of measured ^{238}U/^{204}Pb values in gneisses contrasts strongly with the homogeneous ^{238}U/^{204}Pb value calculated for the source region of the gneiss precursors. Profound uranium depletion is characteristic of many gneisses, yielding present-day unradiogenic Pb isotope ratios close to the primary growth curve[10,11].

By close analogy with the Rb–Sr system, homogeneity of initial Pb isotopes ratios in a gneiss terrain is thus regarded as being inherited from the source region of the gneiss precursors, whereas geochemical differentiation results in dispersion of U–Pb ratios. The necessary conditions for isochron behaviour may thus be met. A good Pb–Pb whole-rock isochron can date the primary accretion-differentiation event, which is usually constrained to a time interval of not more than about 50–100 Myr.

The above interpretation of Rb–Sr and Pb/Pb whole-rock isochron dates in ancient quartzo-feldspathic orthogneisses obviates the necessity for invoking the popular, but highly implausible, process of large scale homogenisation of Sr and Pb isotopes throughout extensive volumes of metamorphic basement rocks.

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Received April 7; accepted April 24, 1975

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Depths of origin of Kenyan basalts and implications for the Gregory Rift

The Gregory Rift of Kenya, part of the eastern branch of the Afro-Arabian rift system, has received much attention in recent years as an exceptionally well exposed example of an intracontinental rift. It exhibits a variety of lavas and ignimbrites related to one another in time and space in patterns that can be clearly defined[1–3]. Explicit models of the crust and upper mantle in and near the Gregory Rift, have been derived, largely from gravity surveys[4,5].

Two petrogenetic models for suites of basalts found in and near the Gregory Rift have been developed. One of these models, for the Chyulu Basalts found east of the rift, overlying Precambrian metamorphic rocks, suggests that the parental basalts of this suite effectively equilibrated with mantle rocks at a temperature of ~ 1,450 °C and pressures of substantially less than 25 kbar[6]. Analytical data on abundances of rare earth elements in the Chyulu Basalts show that assimilation of crustal materials has had at most a very minor effect on the compositions of these rocks, suggesting that the magmas they represent had not remained in contact with crustal rocks for very long. The other model, for members of a suite including a variety of basalts collected in the southern part of the Gregory Rift near Olorgesailie, an area in which the volcanic rocks are predominantly trachytes, suggests that parental basalts of the suite fractionated to produce the more-evolved basalts at temperatures of about 1,200 °C or slightly greater and at pressures of roughly 3–10 kbar (unpublished).

Given the compositions of these basalts, a means of estimating densities of magmas of corresponding compositions at appropriate temperatures and pressures, and models of the densities of rocks from the crust and upper mantle of the region, it is a simple matter to estimate the depths of origin of the magmas if it is assumed that conditions approximating to hydrostatic equilibrium exist. Such estimates provide insight into the structure and geological evolution of the Gregory Rift.

I have taken the compositions of Chyulu Basalt KCH-1 and Gregory Rift Basalt KLR-32 (ref. 6) and computed the densities of liquids with equivalent compositions (according to the method of Bottinga and Weill[7] at 1,400 °C and at 1,250 °C, respectively. The procedure for this is straightforward except that the effects of P_2O_5 and H_2O must be taken into account. I estimated partial molar volumes of P_2O_5 by comparing molar volumes of berlinite and corundum[8], and arrived at a value of about 70 cm^3 mol^{-1} at 1,250 °C and a slightly larger value at 1,400 °C. These estimates are crude but the effect of P_2O on the densities of the liquids is small because of the minor contents of this oxide in these basalts.

The effects of H_2O on the densities are, however, more marked and, because of loss of H_2O from the basalts during eruption and crystallisation, more difficult to assess. There is a high proportion of pyroclastic materials among the products of the Chyulu vents, but there is no evidence for such materials related to the Gregory Rift Basalts treated here. Consequently, it seems likely that the initial H_2O contents of the Chyulu magmas were substantially higher than were those of the KLR basaltic magmas. I have arbitrarily assumed that the initial H_2O content of the KCH-1 magma was 1.0% by mass, and that the initial H_2O content of the KLR-32 magma was 0.5% by mass.

Preliminary calculations indicated that, in agreement with conclusions drawn from petrogenetic models cited already, the Chyulu magmas probably arose from depths great enough to necessitate the application of a small correction for the effects of pressure on the average density, but no correction is necessary for the liquid representing the KLR-32 magma. The estimated densities are 2.71 g cm^{-3} (Chyulu liquid, with pressures ranging from 0–12 kbar) and 2.65 g cm^{-3} (Gregory Rift liquid).

These densities are lower limits for the magmas which gave rise to the analysed basalts; the presence of crystals would tend to increase the magma densities above those estimated under the assumption that only liquid was present. Both basalts are, however, nearly aphyric, and consequently the estimates of densities should not be significantly in error from this cause.

Darracott, Fairhead, and Girdler[5] have devised a model for densities of the lithospheric rocks at various depths near the Gregory Rift: 2.67 g cm^{-3} from 0 to 4 km; 2.85 g cm^{-3} from 4 to 17.5 km; 2.90 g cm^{-3} from 17.5 km to the base of the crust at 36.2 km; and 3.34 g cm^{-3} in the upper mantle down to the base of the lithosphere at about 90 km. The highest peaks of the Chyulu Hills are about 1.4 km above the general level of the surrounding Precambrian rocks. This elevation is probably close to, though somewhat less than, the excess hydrostatic head, h, which governed the eruptions that built up these cinder cones. If h were about 2 km, a column of magma with average density of 2.71 g cm^{-3} would have been in hydrostatic equilibrium with rocks of the model of Darracott, Fairhead, and Girdler at a depth of about 37 km (that is, slightly below the base of the crust), in agreement with the inferences drawn from the petrogenetic model for the Chyulu Basalts[6]. If the volatile content of such a magma column were less than that assumed, or if there were appreciable proportions of crystals suspended in it, its average density would be higher so that h would have to decrease, assuming a constant depth of origin. For instance, if the average density were 2.79 g cm^{-3} as estimated for a volatile-free liquid corresponding to KCH-1, h would be about 1 km if the depth of origin was 37 km. Alternatively, one could assume a slightly greater depth of origin and constant h to account for the effects of a higher density.

These results provide support for the conclusion that the Chyulu magmas originated at unusually high temperatures and (for alkaline basalts) low pressures, and also increase confidence in the model of Darracott, Fairhead, and Girdler[5] and in the Bottinga and Weill[7] method for estimating magma densities.

Consider applications of similar arguments to the basalts of the Gregory Rift near Olorgesailie. If the KLR-32 magma, with a predicted density of 2.65 g cm^{-3}, erupted from a depth of origin, h, of 1 km, it could have been in hydrostatic equilibrium with its wall rocks as represented by the model of Darracott, Fairhead, and Girdler[5] only if it had its roots about 13 or 14 km below the rift floor. Probably, KLR-32 does not represent a relatively unmodified mantle-derived magma, but instead was stored (and presumably fractionated) in a shallow magma chamber within the axial zone of the rift before eruption.

Some characteristics of this putative, shallow magma chamber can be inferred from the results of geophysical studies of the rift. In 1970, Searle (ref. 9) modelled the gravity anomaly along the axis of the central Gregory Rift as a large basic intrusion, with a density of 2.90 g cm^{-3} at depths of less than about 25 km, which penetrates to within about 3 or 4 km of the floor of the rift. This conclusion was accepted in slightly modified form by subsequent workers[4,5], and it leads to a plausible model for the tectonics of the rift. Of course, there is no reason to believe that this dense intrusive body was produced by the crystallisation of a single, large magma reservoir rather than by crystallisation of a series of smaller magmatic injections, nor does the geophysical evidence require that the intrusive body be in contact with the upper mantle.

If magma which crystallised to form a part of this intrusive body were present in the axial zone of the Gregory Rift near Olorgesailie at the time of the eruption of KLR-32 about 1.6 Myr ago, it should have been in hydrostatic equilibrium with its wallrocks. Fixing its top at 3 km below the rift floor and assuming that it was in effective contact with wallrocks like those modelled by Darracott, Fairhead and Girdler, its density must have been appreciably greater than that estimated for the KLR-32 magma; it perhaps resembled the KCH-1 magma in that respect. It cannot have had vertical dimensions greater than about 10–20 km: had that been the case eruptive basaltic volcanism would have been much more common in the area at that time. This magma may have been a thin veneer on top of dense intrusive rocks representing the solidified remains of similar, older magmas related to previous cycles of basaltic volcanism (the Singaraini, Kordyja, and Kirikiti basalts).

It seems that we are dealing with something like a bubble of magma, or a series of such bubbles, drawn off from the upper mantle either in response to, or as a cause of, rifting and confined to the axial zone of the rift, but of limited vertical extent. Note that the crustal thickness of about 36 km in this region would have allowed a much bigger bubble to be confined in the axial zone of the rift. A bigger bubble would necessarily have manifested itself in a phase of extensive basaltic effusive volcanism, as is observed further north in the Gregory Rift[1–3]. Probably, the distinction between portions of the rift in which basaltic flows predominate and those in which basalts are uncommon and trachytic or phonolitic flows predominate is simply a reflection of different vertical dimensions of the axial bubbles, which are in turn related either to the volume of basaltic magma that may be drawn off from the underlying upper mantle or to the propensity of the mantle for giving up its magma in one large batch or in two or more smaller batches.

Presumably, contrasts in the volume of basaltic magma that may be derived in a short interval from a given area of the upper mantle under the rift are related to the fertility of the mantle, with those portions of the upper mantle that have previously been physically depleted being incapable of generating enough basaltic magma, or of generating it fast enough, to provide a big bubble. In this context, it is interesting that basaltic volcanism seems to die away as the rift continues into the African continent. To the degree that the suboceanic upper mantle participates in the plate tectonic cycle, it may be replenished with the components that are required for the generation of basaltic magma and so remain capable of producing magma, but subcontinental upper mantle that is part of the lithosphere may be more or less isolated from the plate tectonic cycle and so rather easily becomes depleted of magma-producing constituents. Perhaps the different styles of volcanism at different locations along the Gregory Rift reflect a broad transition from one kind of upper mantle to another. These speculations may be tested, *inter alia*, by studies of Sr and Pb isotopes in rocks collected from portions of the Gregory Rift which show different styles of volcanism.

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Shatter cones from Haughton Dome, Devon Island, Canada

SHATTER cones, products of shock metamorphism, have been discovered at Haughton Dome and indicate a meteorite impact origin for this circular structure. Haughton Dome is centred at 89°40′W, 75°22′N on Devon Island in the Canadian Arctic Archipelago. Although structurally a dome, it forms a topographic depression incised on the regional plateau (Fig. 1). The inner, circular basin, 17–18 km diameter, has an average elevation of 140 m above sea level compared with regional elevations of 340–400 m. It is surrounded by an annular ridge with elevations averaging 270 m above sea level. In the southern portion of the structure the ridge is not pronounced, but forms a shoulder on the gradual upward slope to the regional plateau, approximately 17 km from the centre. Streams draining the basin have dissected the ridge in the northern portion into an arc of isolated hills. Steep-walled drainage valleys outside the ridge are circumferential or tangential to the structure and enhance its circular appearance.

The Precambrian basement of Devon Island is exposed 100 km to the east, and its surface forms a gently westerly dipping homocline[1]. Exposures of the overlying sedimentary sequence proceed in westerly succession from Cambrian (?) to Devonian (?) with the Allen Bay Formation (Upper Ordovician to Middle Silurian) forming the plateau in the Haughton Dome region. Dolomite, limestone and gypsiferous and dolomitic shales of the Cornwallis Group (M. Ord.) underlie the Allen Bay limestones and are exposed at the base of cliffs along Thomas Lee Inlet and in river valleys 10–15 km east of Haughton Dome, where beds dip westward at less than 2°.

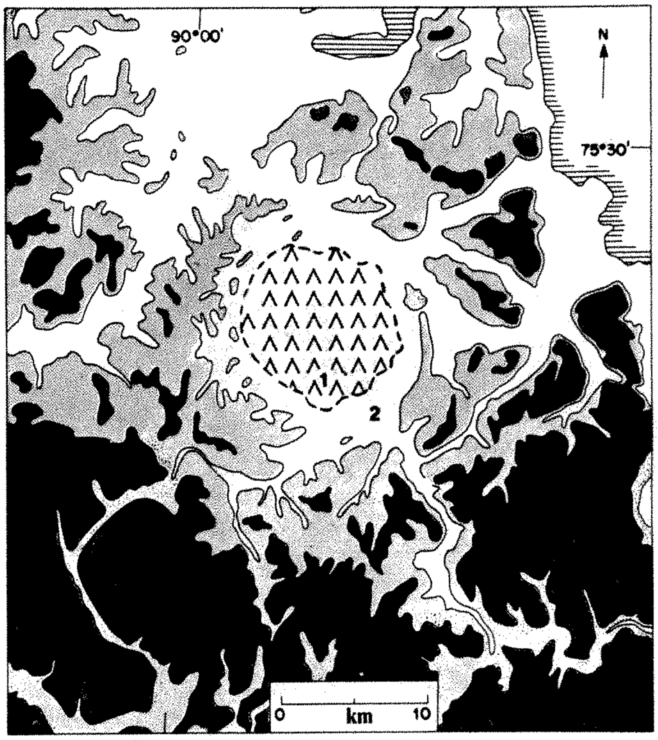

Fig. 1 Topographic map of the Haughton Dome structure showing central depression and partial annular ridge. Morphology of the structure is enhanced by leaving unshaded elevations below 800 feet (244 m) above sea level. Thereafter, shading darkens with each 200 feet (61 m) increase in elevation. Horizontal shading is Thomas Lee Inlet. ΛΛΛ, Approximate outcrop area of the evaporite unit. Shatter cones were collected in limestone rubble at location 1, but were not found at 2.

The geology of Haughton Dome is partially known from mapping in the south-east quadrant[2], supplemented by aerial reconnaissance and examination of aerial photographs. Exposed in the central depression are limestones and evaporites which form white cliffs 60–80 m high. Greiner[2] observed complex folds and zones of shearing in this area. The central depression is surrounded by the following succession, as mapped in the south-east[2]: a zone of brecciated limestone; a zone of "complex structures and lithology" which may form the annular ridge; limestones and dolomites in a series of shallow anticlines and synclines with axes tangential to the structure at approximately 10 km from the centre. Correlation between regional stratigraphy and disturbed rock units within the structure is tenuous. Greiner[2] considered the evaporites to be the lower part of the Cornwallis Group, which does comprise a thick evaporite section on islands 140 km west and north[1], but not in this part of Devon Island. More probably, from their weathering characteristics, the evaporites may correlate with the Lower Ordovician Baumann Fiord Formation which is widespread in the eastern Arctic islands[3], a formation which was not known when Greiner made his correlation. Limestones and dolomites of the marginal zones include Cornwallis and Allen Bay units, and possibly the Eleanor River Formation which separates the Baumann Fiord and Cornwallis Group. Because of the circular exposure of evaporite surrounded by younger disturbed rocks, Haughton Dome has been considered a piercement dome[1], especially as many piercement domes are emplaced in the Sverdrup Basin to the north[4]. These structures are, however, smaller and form a distinct cluster in a different tectonic setting 400 km distant.

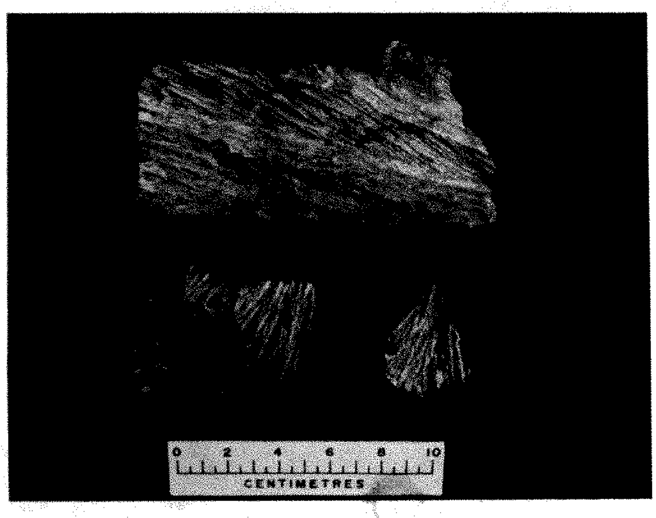

Fig. 2 Shatter cone fragments in fine-grained limestone. Other fragments indicate cones at least 30 cm high.

Shatter coning and intense microtwinning of calcite, present in samples from Haughton Dome, are indicators of shock metamorphism. Shatter cones are abundant in limestone rubble at the one locality visited within the central depression (Fig. 1). Their extent has not been determined although shatter cones were not found at a locality in the outer brecciated limestone zone. Most of the samples collected (by G.D.M.) are broken, partial cone surfaces (Fig. 2) with 110° the maximum arc found intact. Shatter cones on surfaces where apex and base are seen are generally 4–7 cm high, whereas surface curvature and convergence of striations on broken segments indicate cones at least 30 cm in height. The characteristic horsetail ridges are finely-developed on the fine-grained limestones, although erosion or a skin of calcite plus aragonite commonly subdues or masks all but the coarser striations.

Shatter cones are accepted as products of hypervelocity impact because of their occurrence only at meteorite craters and astroblemes[5]. They are formed by tensional release in an axially symmetrical field following compression by an expanding shock wave[6] with peak pressures in excess of approximately 2 GN m^{-2} (1 Gnewton $m^{-2} = 10$ kbar). The absence of shatter cones at locality 2, 8 km from the centre, is consistent with their disappearance at approximately one-half radius in large impact structures[7].

Calcite in the shatter-coned limestones shows intensive microtwinning with up to six sets per grain. The twin planes, $(01\bar{1}2)$ and $(10\bar{1}1)$, are those commonly produced in tectonic deformation, but their close spacing, $< 20\ \mu m$, is consistent with shock metamorphism as illustrated in shatter-coned limestones at the Flynn Creek crater, Tennessee[8]. Shock-induced planar features were not observed in the minor amounts of detrital quartz grains within the Haughton Dome limestone samples.

Haughton Dome can be classed as an eroded, complex, hypervelocity impact structure with a central structural uplift[9]. It can be compared with Sierra Madera (12 km) and Wells Creek (13 km) structures, formed largely in carbonate rocks. At these two impact sites material in the central uplift has been raised 1,200 m (ref. 10) and 780 m (ref. 11) respectively. Based on average stratigraphic thicknesses and regional dips, uplift at Haughton Dome is approximately 400 m (if Cornwallis evaporite in centre) to 1,500 m (if Baumann Fiord evaporite in centre). The latter figure, of a magnitude more consistent with uplift at Sierra Madera and Wells Creek, tends to support a correlation of the central evaporite with the Baumann Fiord Formation.

From stratigraphic constraints the maximum age of the Haughton Dome impact is 425 Myr (that is younger than the Allen Bay Formation) although it predates Pleistocene glaciation.

The original suggestion of Dence[9] that Haughton Dome is an impact structure is confirmed by the evidence of shock metamorphism in the form of shatter cones. The addition of Haughton Dome brings the number of confirmed meteorite impact structures recognised in Canada[12] to twenty-one.

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Received March 18; accepted April 16, 1975.

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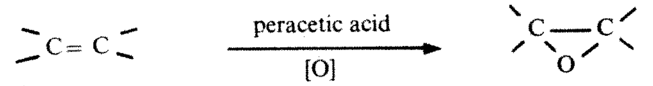
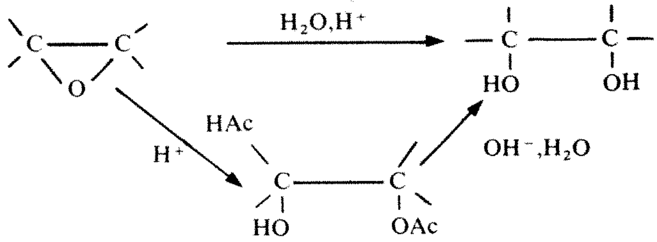
Effect of stress transfer modified swelling on diffusion in polymers

We have noticed that if certain 'immobile' triblock copolymers of the styrene–butadiene–styrene (SBS) type are subjected to surface hydroxylation by peracetic acid (a low molecular weight diffusing species) some anomalies occur in the diffusion–reaction process. We attributed these anomalies to the previously unnoticed effect of swelling in the surface region of the SBS polymer, which in turn was modified by the transfer of the swelling stresses to the interior during the reaction.

To hydroxylate SBS block copolymers in the bulk state, films of the copolymer (Shell experimental block copolymer TR-41-2443; Mn = 118,000; 27.7 weight % polystyrene) of various thickness were suspended in a reaction bath containing peracetic acid, sulphuric acid and varying amounts of acetic acid and water. The peracetic acid, after diffusing into the polymer, reacts with the double bonds in the polybutadiene continuous phase of the film to form an epoxide product:



The epoxide product then reacts with water or acetic acid, with sulphuric acid as a catalyst, to form, respectively, a 1,2 glycol or a hydroxyacetate addition product, the acetate group of which is hydolysed by a base in a separate bath to give a uniform product:



The cleavage agents (including sulphuric acid) must also diffuse through the polymer to effect the ring opening of the epoxide groups.

The diffusion of all species into the polymer is governed by their respective diffusivities and solubilities in the polymer. On epoxidation, the polymer is converted from a hydrophobic to a

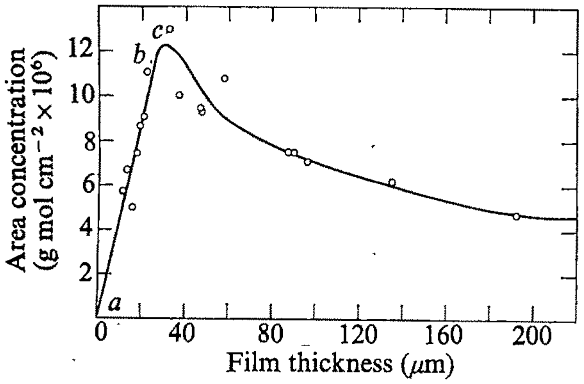


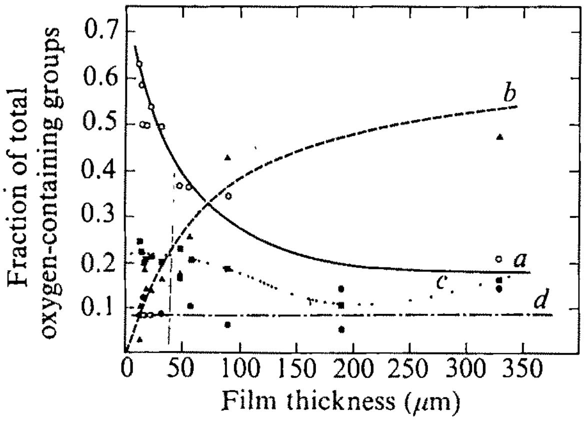
Fig. 1 Area concentration of hydroxyl against film thickness; 30 °C, 180 min, 40% acetic acid. The area concentration of hydroxyl groups, measured by infrared spectroscopy of intact reacted film, is defined as $\int_0^b C_{OH}$ (b = film thickness in cm; C_{OH} = local concentration of hydroxyl groups, mol cm^{-3}) and is the total number of moles of hydroxyl groups present in the film per unit area.

hydrophilic material and the solubilities of the diffusing species are increased. In addition, swelling occurs simultaneously with reaction, resulting in an increase in the diffusivity and a still further increase in solubility. These increases of diffusivity and solubility are, however, modified by the presence of an elastic network in the SBS film, which acts to transfer the swelling stresses to the unreacted and, therefore, unswollen core (see ref. 1). This transfer of stress acts to reduce the degree of swelling in the surface region and limits the increases of diffusivity and solubility. As the degree of stress transfer depends directly on the relative size of the unreacted core and the reacted surface region, the thickness of the film has a strong effect on the permeation rate of peracetic acid and on the cleavage agents, acetic acid and sulphuric acid (resulting in a lowered permeation rate in thicker films than in thinner films).

Contrary to expectations the extent of reaction did not become independent of film thickness at a particular depth of penetration (Fig. 1). Similar observations—an initial linear increase in area concentration (extent of reaction) followed by a gradual decrease beyond a certain maximum value (Fig. 1)—were recorded for each set of conditions studied.

There are two reasons for the maximum at c in Fig. 1 and for the subsequent decrease. Quantitative analysis of the spectra of the films show the same decrease in hydroxyl concentration and a commensurate increase in epoxide content (Fig. 2). Because of the transfer of stress to the dry interior, the surface regions of the thinner films swell more than those of the thicker films, enabling the slower diffusing epoxide cleavage agents (acetic acid, water and sulphuric acid) to diffuse more effectively into the thinner films, thus reducing the lag that exists between the advancing concentration profiles of peracetic acid and the cleavage agents. As a result, cleavage of the epoxy rings is more nearly complete in thinner films than in thicker films, and yields higher hydroxyl contents.

Fig. 2 Composition of surface hydroxylated films; 30 °C, 180 min, 70% acetic acid. The concentrations are reported as the fraction $C_i/\Sigma C_i$, where ΣC_i = sum of equivalents of all oxygen containing species. C_i determined by infrared spectroscopy of ground samples of reacted films. a and ○, Hydroxyl; b and ▲, epoxy; c and ■, cyclic ether; d and ●, acyclic ether.



As the degree of reaction increases, the amount of swelling becomes sufficient to reduce the lag between the two concentration profiles to the extent that, apparently, the product distribution is no longer dependent upon the thickness of the film. Nevertheless, the hydroxyl content still decreases with increasing film thickness. We attribute this to the effect of stress-transfer modified swelling in the surface region on the depth of penetration of the epoxidation reaction itself. The lowered rate of permeation of peracetic acid in the thicker films (because of the reduced degree of swelling) results in a smaller depth of penetration of the reaction zone in thicker films, which is directly related to the lower hydroxyl content in these films even where there is no change in the product distribution.

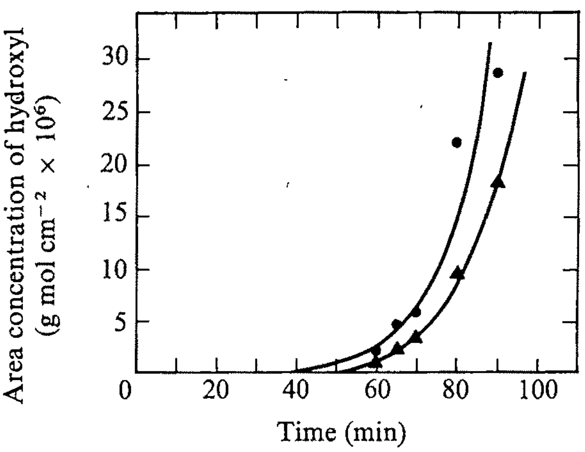


Fig. 3 Time course of surface hydroxylation. Area concentration of hydroxyl against time; 40 °C, 71% acetic acid. ●, 60 μm; ▲, 300 μm.

The exponential time course of the surface hydroxylation process and the apparent induction time of approximately 50 min (for a reaction at 40 °C) which lapses before there is any evidence of hydroxyl groups in the infrared spectrum are both evident in Fig. 3. The depth of penetration is, therefore, less than 0.2–0.3 μm (as calculated from the OH absorptivity and the minimum peak area distinguishable in the spectrum) with 50 min of reaction, but is increased to a depth of the order of 20–25 μm—a 100-increase—in just 15 min extra. This apparent induction time is related directly to the exponential nature of the process.

This exponential relationship could also be a result of swelling in the surface region. As the surface region swells, the solubility of the peracetic acid in the polymer increases and the driving force for diffusion is increased commensurately. Thus, the diffusion rate increases as the depth of penetration increases and as the degree of swelling increases at the surface because of the relief of the limiting compressive stresses. This further increases the peracetic acid solubility. This 'autoacceleration' behaviour can be verified by increasing the concentration of acetic acid in the reaction bath. Acetic acid, being a better swelling agent for the reacted polymer, should lower the length of the induction period as the same solubility should be attained at an earlier time; in fact the extent of reaction is greater after only 20 min in 93% acetic acid than it is after 65 min in the 71% acetic acid.

Surface hydroxylation is, therefore, a combined reaction limited and diffusion limited process, modified by the simultaneous swelling of the reacted surface region, which in turn is limited by stress transfer to the unreacted core. This unusual and unexpected behaviour, in which the film thickness has an effect on the diffusion and subsequent reaction of a low molecular weight permeant with the film is believed to be a general phenomenon characteristic of a variety of situations involving interfacial conversion including, for example, the oxidative photodegradation of polyethylene.

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Received December 27, 1974; accepted March 5, 1975.

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Disorder in a hydrophobic cage illustrated by X-ray structure of α-cyclodextrin·methanol pentahydrate adduct

α-Cyclodextrin (α-CD, cyclohexaamylose) is a cyclic molecule consisting of six α(1→4) linked glucose residues. Because of its annular structure it can form inclusion complexes with a wide variety of guest molecules even in aqueous solution and has been studied as an enzyme model[1]. The structure of the 'empty' water adduct suggests a release of conformational and intramolecular hydrogen bonding energy of the α-CD molecule when complex formation occurs[2,3]. To study this process in more detail, we have investigated the adduct with methanol.

The α-CD·MeOH adduct was crystallised through slow cooling of a saturated solution of α-CD in 50% aqueous methanol. The space group of the crystals is orthorhombic, $P2_12_12_1$, with unit cell dimensions $a = 14.339$ Å, $b = 37.365$ Å and $c = 9.465$ Å. The intensities of 4,283 reflections were measured using a STOE four circle diffractometer with Ni-filtered Cu K_α-radiation and a $\theta/2\theta$ scan with a maximum 2θ of 120°.

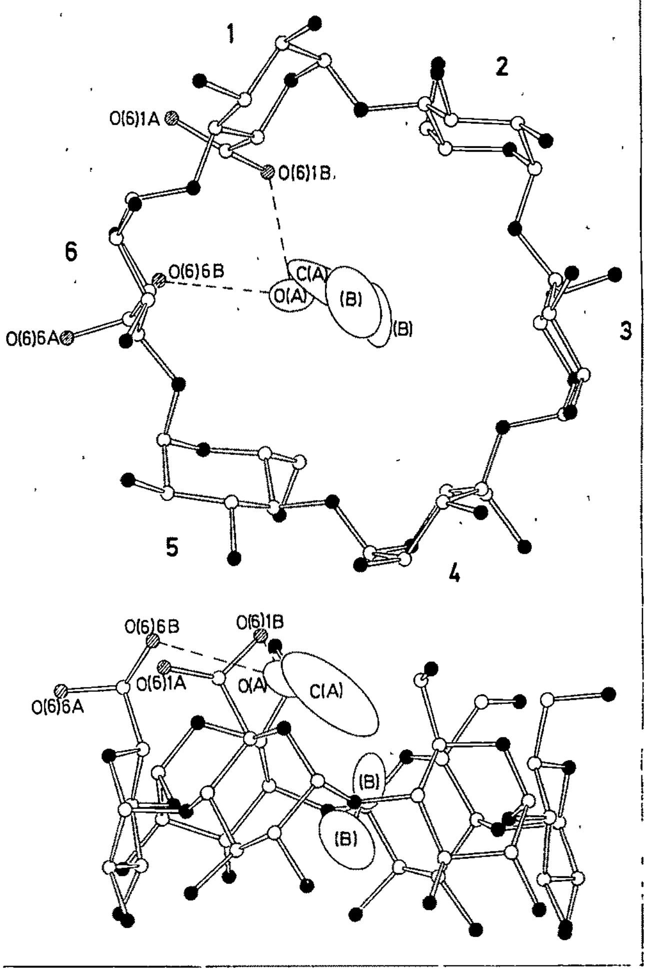
The structure was solved by assuming it to be isomorphous with the recently published α-CD·*n*-propanol·4.8 hydrate complex[4] which is also orthorhombic, $P2_12_12_1$ with cell dimensions $a = 14.292$ Å, $b = 37.515$ Å, $c = 9.393$ Å. The atomic parameters of the α-CD·MeOH pentahydrate structure were refined by full matrix least-squares methods to a conventional discrepancy index $R = 4.3\%$ for all the measured data. Positions of hydrogen atoms were determined from Fourier difference syntheses, hydrogen atoms bonded to disordered hydroxyl or methyl groups were not located.

As expected, the structure of the α-CD·methanol adduct is similar to that of the *n*-propanol adduct (Fig. 1). In both the *n*-propanol and methanol adducts, atom O(6)6 (the O(6) hydroxyl group of glucose number 6) has twofold disorder, but in the methanol adduct, O(6)1 is twofold disordered as well. The methanol hydroxyl group O(A) is present at the same position as the *n*-propanol hydroxyl group in the *n*-propanol adduct with the other parts of the alcohol molecules being in different locations. The conformation of the α-CD molecule in the methanol adduct is quite similar to that in the *n*-propanol adduct. The O(4) atoms are nearly coplanar with a maximum deviation of 0.17 Å from the common mean plane similar to the *n*-propanol structure as well as the α-CD-potassium acetate[5] and α-CD·I_2 structures[6], but different from the strained conformation of the α-CD molecule in the water adduct[2,3]. The α-CD macrocycle is not as symmetrical as in the *n*-propanol adduct, since the O(4) · · · O(4) distances have a wider variance, being 8.25, 8.44 and 8.66 Å, while in the *n*-propanol adduct they are 8.35, 8.47 and 8.57 Å. This discrepancy may result from the disorder of atom O(6)1 in the methanol adduct. Both hydroxyl groups O(6)6B and O(6)1B are pointing in towards the centre of the α-CD molecule and make close contacts with O(A) of the methanol. Along this direction the molecule contracts to 8.25 Å for the O(4)3 · · · O(4)6 distance. The larger substrate molecule, *n*-propanol, apparently enables the α-CD molecule to be more symmetrical and less strained, while the smaller methanol molecule leaves α-CD slightly more strained. Going from water to methanol to propanol to potassium acetate the conformation becomes more and more symmetrical and less strained with the water adduct being substantially different from any other inclusion complex.

The two methanol molecules in the α-CD·methanol adduct are seen with half populations. The C(A)–O(A) molecule is nearly in the plane of the O(6) atoms, although C(A) has a high elongated vibrational ellipsoid. O(A) has close contacts with O(6)6B and O(6)1B as well as with O(2)1 of a neighbouring molecule. The methanol molecule (B)–(B) is nearly coincident with the axis of the α-CD molecule and is centred closer to the O(2), O(3) side. It has a high temperature factor and is held only by van der Waals' forces.

As most of the hydroxyl hydrogen atoms have been located, the hydrogen bonding scheme can be summarised. The regular hydrogen bonds between the O(2) and O(3) atoms of adjacent glucose residues are confirmed by the presence of the H atoms. The O(2) · · · O(3) distances are 3.07, 2.96, 2.91, 2.96, 2.95 and

Fig. 1 The structure of the α-cyclodextrin · methanol pentahydrate complex. The glucose numbering scheme used in the text is indicated. The two included methanol molecules O(A)–C(A) and (B)–(B) are shown with half population each and drawn with their 50% probability thermal ellipsoids. In methanol molecule A, the hydroxyl group O(A) is hydrogen bonded to O(6)6B, O(6)1B (dashed lines) while molecule B is held by van der Waals' forces and C,O atoms could not be assigned with certainty.



3.15 Å. Even for the distances longer than 3 Å the atoms were found with good O–H · · · O angles between 160° and 180°. Although this could not be seen in the *n*-propanol adduct as the hydroxyl atoms were not found, it is consistent with the potassium acetate adduct structure. Other than these regular features, all the hydroxyl groups O(2), O(3) and O(6) form hydrogen bonds to either water molecules or atoms of adjacent cyclodextrin molecules.

The structure of the α-CD molecule in the α-CD·MeOH complex and in the recently investigated α-CD·krypton complex[7] is different from that in the α-CD·water complex with composition α-CD·$2H_2O$. With the methanol and the krypton substrate the α-CD molecule is almost hexagonal in shape and the methanol and krypton molecules are highly disordered to occupy the 5 Å wide cavity. But with the H_2O guest molecules the α-CD cavity is decreased by an inward rotation of two glucose residues to hold the two H_2O molecules in van der Waals' contact and the H_2O molecules are further fixed through hydrogen bonding. The inward rotation of the two glucose residues in the water adduct causes a conformation with increased strain energy and disrupted intramolecular O(2) · · · O(3) hydrogen bonds between adjacent glucoses[3] and explains why in aqueous solution α-CD is able to form adducts with such a variety of guest molecules: adduct formation occurs through release of conformational strain energy of the α-CD molecule and formation of continuous O(2) · · · O(3) intramolecular hydrogen bonds between adjacent glucoses[3,7].

B.H. thanks the National Science Foundation for a NATO postdoctoral fellowship. All computations were carried out with the UNIVAC 1108 computer at the computing centre of the Gesellschaft für wissenschaftliche Datenverarbeitung, Göttingen, and were supported in part by the Deutsche Forschungsgemeinschaft.

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Continental drift and the use of albumin as an evolutionary clock

ACCORDING to the concept of proteins as evolutionary clocks[1], change in amino acid sequence during evolution is primarily a time-dependent process. This process accounts for the correlation generally found between the amount of time that has elapsed since two species last shared a common ancestor and the degree to which the sequences of their proteins differ today. A body of data consistent with such a correlation is now available for many proteins and nucleic acids[2–9]. Although there is increasingly widespread agreement that such a correlation exists[10–12] it is difficult to ascertain how strong the correlation is because of uncertainties in the interpretation of the fossil record, particularly with regard to times of divergence of lineages.

An alternative source of information about divergence times is sometimes available, even for taxonomic groups that have extremely poor fossil records: such information comes from studies of continental drift. When the land bridge between two continents disappears, many of the species on one continent become genetically isolated from their relatives on the other continent. By making intercontinental comparisons of the proteins of those taxonomic groups which were separated by continental drift, one can test the evolutionary clock hypothesis. For the intercontinental tests described here, we use serum albumin.

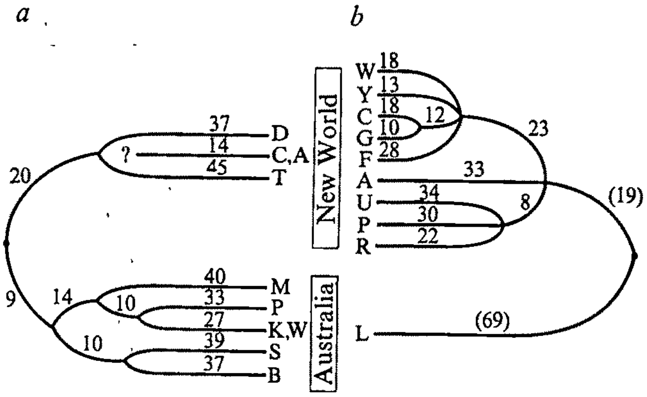

Fig. 1 Phylogenetic relationships among the albumins of marsupials (*a*) and hyline tree frogs (*b*) from Australia and the New World. For the methods of tree construction used, see refs 17, 18 and 29. By comparing immunologically each of the marsupial albumins with those of selected placentals (*Hyaena, Ursus, Genetta*), we found that *Caluromys* and *Marmosa* albumins are more conservative (that is, react more strongly with the anti-placental albumin sera), by 25±5 units, than those of the other seven lineages of New World and Australian marsupials studied. The numbers above the lineages indicate the number of albumin immunological distance units of evolution calculated to have occurred along each lineage. The position of the *Caluromys* and *Marmosa* lineage(s) is not clearly defined by the available data. D, *Didelphis*; C,A, *Caluromys* and *Marmosa*; T, *Metachirus*; M, Macropodidae; P, Phalangeridae; K,W, Koala and wombat; S, Dasyuridae; B, Peramelidae; W, *H. wrightorum*; Y, *H. chrysoscelis*; C, *H. cinerea*; G, *H. gratiosa*; F, *H. femoralis*; A, *Acris crepitans*; U, *H. crucifer*; P, *Pseudacris triseriata*; R, *H. regilla*; L, *Litoria aurea*. A fuller presentation of the hyline phylogeny is made in ref. 29.

Quantitative immunological comparisons indicate that the various albumin lineages have accumulated amino acid substitutions with considerable regularity[13–20], although in birds[20] the rate is two or three times lower than in other vertebrates tested. We have been impressed with fragmentary evidence which suggests that the rate of albumin evolution in frogs[16,18] is similar to that in placental mammals, in spite of the fact that evolution at the organismal level has been slower, by far, in frogs than in mammals[21–22]. Because the fossil record of frogs is so inadequate, however, it was difficult to know how similar the rates of albumin evolution have been in the two groups. We therefore sought an alternative approach to the problem of comparing the rates of albumin evolution in organisms with poor fossil records. The problem is of interest not only from the standpoint of trying to understand the mechanisms of protein evolution, but also because albumin and other proteins may prove to be useful evolutionary dating devices.

Recent studies of continental drift provide an appropriate zoogeographical setting to test the albumin clock hypothesis. We have considered the mammal superorder Marsupialia and the tree frog subfamily Hylinae, both of which have a distribution which includes the New World and Australia and excludes Africa and South-east Asia. Studies of global tectonics indicate that South America and Australia were relatively recently connected by way of Antarctica[23–25]. Thus if the marsupials and tree frogs have a southern continent ancestry, the most recent existence of those proposed ancestors must predate the separation of the two continents. Indeed, disappearance of the trans-Antarctic bridge may have been the event which separated the marsupial and hyline lineages represented today in Australia from those in the New World[26].

Albumins were purified from 17 marsupial species (13 Australian, 4 New World) and 10 hylines (9 New World and

Table 1 Immunological comparison of Australian and New World albumins

Taxa compared		Immunological distances‡ Anti-A against B	Anti-B against A	Mean
	Marsupials*			
A	B			
Macropodidae	*Caluromys, Marmosa*	90	94	92
Macropodidae	*Didelphis, Metachirus*	115	118	116
Phalangeridae	*Caluromys, Marmosa*	91	94	92
Phalangeridae	*Didelphis, Metachirus*	133	131	132
Wombat, Koala	*Caluromys, Marmosa*	84	84	84
Wombat, Koala	*Didelphis, Metachirus*	120	117	118
Dasyuridae	*Caluromys, Marmosa*	82	79	80
Dasyuridae	*Didelphis, Metachirus*	125	119	122
Peramelidae	*Caluromys, Marmosa*	80	79	80
Peramelidae	*Didelphis, Metachirus*	116	119	117
Mean for comparisons involving *Caluromys* and *Marmosa*		85	86	86
Mean for comparisons involving *Didelphis* and *Metachirus*		122	121	121
Mean for marsupials		103	103	103
	Hylines†			
A	B			
Litoria	*Hyla wrightorum*	158	113	136
Litoria	*H. chrysoscelis*	130	103	116
Litoria	*H. cineria*	158	146	152
Litoria	*H. gratiosa*	150	93	122
Litoria	*H. femoralis*	163	122	142
Litoria	*Acris crepitans*	105	121	113
Litoria	*H. crucifer*	141	137	139
Litoria	*Pseudacris triseriata*	156	102	129
Litoria	*H. regilla*	129	97	113
Mean for hylines		144	114	129

*Antisera to the purified albumins of two or more Australian genera in each group and of the four New World genera (*Didelphis, Caluromys, Marmosa, Metachirus*) were used to measure the various immunological distances between the albumins of the respective Australian groups and those of the New World forms. Macropodidae included *Bettongia, Macropus*, and *Dendrolagus;* Phalangeridae included *Phalanger, Petaurus*, and *Pseudocheirus;* Dasyuridae included *Dasyurus, Dasyuroidea*, and *Myrmecobius;* and the Peramelidae were *Echymipera* and *Isoodon*.

†Antisera to the purified albumins of the Australian *Litoria aurea* and all the New World species listed were used in these tests A fuller presentation of the results of these tests is given in ref. 29.

‡For each pair of species or groups of species two values are given because reciprocal tests were made (that is, anti-A with B and anti-B with A); the last column gives the average of the two.

1 Australian). Antisera to each albumin were prepared by immunising a group of three to five rabbits for 3 months. The methods of purification and immunisation are described elsewhere[17,18,27]. The antisera to a given albumin were then pooled in inverse proportion to their micro-complement fixation (MCF) titres, and antigenic differences among the various albumins measured by the MCF procedure using these pools[28]. The degree of antigenic difference between any two albumins is expressed as an immunological distance[28] where, for albumin, one immunological distance unit is equivalent to about one amino acid substitution[27].

Table 1 shows that the mean immunological distances between the Australian and New World albumins are quite similar, 103 for marsupials and 129 for tree frogs. As the distances range from 80 to 152, however, it seems that albumin evolution has been quite irregular in both the marsupials and hylines. Thus evolutionary change in the antigenic properties of the albumin molecule in one hyline pair may have developed at almost twice the rate of one marsupial pair. Although the presence of such discrepancies precludes any naive use of an albumin clock, we think that the divergence events at issue can be dated provided the data are properly analysed.

The basic precaution should be to separate out the nonrandom discrepancies from the random variations which are a component of any non-deterministic clock. The first of these is evident in the marsupial data where the mean immunological distance from the Australian species to *Didelphis* and *Metachirus* is 121 units, whereas that to *Caluromys* and *Marmosa* is only 86 units. The significance of this result does not become evident until a phylogenetic (cladistic) analysis is carried out using all possible intra-Australian and intra-American comparisons in addition to the intercontinental distances given in Table 1. The results of this analysis are shown in Fig. 1 and indicate first that those species studied on a given continent form monophyletic groups relative to those on the other. The marsupial analysis is facilitated because we are able to use placental mammal albumins as outside reference points to estimate the relative amounts of change along the various marsupial albumin lineages; that is, we assume that the living marsupials share common ancestry subsequent to the divergence of the ancestral placental lineage. As Fig. 1 shows, the lineage(s) leading to *Caluromys* and *Marmosa* has experienced much less albumin change than those of the other marsupials, for whom the amount of albumin change since the separation of the Australian and New World groups is about 61±5 units. Note that the existence of an albumin clock cannot be assumed; it must be documented for the particular group being studied Thus, times of divergence are calculated by first allocating the amount of albumin change along the various lineages involved, noting if these are similar enough to be compatible with the clock model, and then using the mean amount of change along those lineages consistent with the clock model as a measure of their age. Thus the marked deceleration in albumin change for *Caluromys* and *Marmosa* is clearly indicated by internal analysis and is ignored in dating the separation of the two marsupial groups.

The hyline data illustrate a problem specifically associated with the immunological approach The anti-*Litoria* values in Table 1 are generally higher than those obtained in reciprocal tests. This is not a unique occurrence as similar degrees of variations exist for a small percentage of the hundreds of antisera prepared in this laboratory. Thus, just as the mean immunological distance between albumins of the Australian and New World marsupials underestimates the actual phyletic distance (because of the lack of change along the *Caluromys* and *Marmosa* lineages), so too is the corresponding hyline distance perhaps overestimated by giving the anti-*Litoria* values (mean of 144) a weight equal to the nine New World antisera combined (mean 114). To check this, antisera to additional Australian hyline albumins are needed, but until these are prepared it is probably best to accept 114 units as the best available estimate of the intercontinental hyline distance.

The rate constant for albumin evolution in placental mammals and iguanid lizards is about 1.7 units per Myr per pair of lineages compared[13–15,17,18,30]. If this holds for marsupials and hylines, then for the separation of the Australian and New World faunas, one obtains a figure of 73 Myr for marsupials and 68 Myr for hylines. These figures are consistent with current geological dating of the South America–Antarctica separation as occurring in the late Cretaceous 70 Myr ago[23–25].

Thus the sources of our discrepancies have been identified by internal analysis. For the resulting level of accord to exist, the albumins of two groups differing enormously in their histories, ways of life, and rates of morphological evolution must have accumulated approximately equal amounts of immunological change over 70 Myr. These differing rates of morphological evolution are reflected in the formal taxonomies of the groups—all the frogs studied being in the subfamily Hylinae, whereas it takes a superorder to encompass the marsupials. Indeed, until 1971 *Litoria* was included in the genus *Hyla*[31]. Secondly, for the immunological approach to produce such consistent results, it must be measuring something which is evolving in a regular fashion. This something is presumably surface amino acid substitutions in the several albumin lineages being studied. Direct evidence from immunological studies of proteins of known sequence and configuration indicates that this is the case[32–34]. Thirdly, the calibration of the albumin clock at 100 units of immunological distance accumulated per 60 Myr of separation provides dates which agree with the latest global tectonics evidence and with vital zoogeographical considerations. If South America and Australia had been connected during the early Tertiary, then one would expect to find evidence of ancient placental lineages in Australia—there is none. On the other hand, if the trans-Antarctic connection had ceased to exist much earlier in time than the late Cretaceous, then one could not have had the appropriate marsupial ancestors in South America in time to get them over to Australia. Finally, the congruence of the marsupial and hyline tree frog pictures discussed here provides in itself strong support for the proposed trans-Antarctic passage of the two groups to Australia—a matter still in some dispute for at least the marsupials[35].

Although the disappearance of the trans-Antarctic connection was probably the event which separated the marsupial and hyline lineages existing today in Australia from those in the New World, an alternative should be considered. One or both of the divergences may, in theory, have occurred well before the continents drifted apart. A more ancient divergence coupled with slower albumin evolution would be compatible with the results given above. This alternative is unlikely, however, in view of the phylogenetic position of marsupials within the class Mammalia and of the hylines within the superfamily Bufonoidea. The mean albumin immunological distance between marsupial and placental mammals is about 200 units, which corresponds to an early Cretaceous divergence, in agreement with palaeontological opinion[36,37] as well as with sequence evidence derived from haemoglobin and myoglobin[7]. These considerations also suggest that deceleration in albumin change occurs in *Caluromys* and *Marmosa*, rather than acceleration in the other marsupial albumin lineages studied. Similarly, our intra-bufonoid studies[29] indicate maximum albumin immunological distances of about 180 units within that group, which is consistent with other evidence[38,39] that the bufonoid frogs arose in South America shortly after its separation from Africa, that is, in the middle Cretaceous period. Thus, consideration of the evolution of hylines and marsupials within a larger taxonomic context does not suggest that albumin evolution has been significantly retarded in marsupials or hylines. We infer, therefore, that the lineages leading to living Australian marsupials and hylines diverged from those leading to their New World counterparts at a time that did not significantly predate the disappearance of the trans-Antarctic land bridge between these two regions.

Even though the albumin clock may not always keep perfect time, it retains considerable potential for evolutionary dating. For that potential to be fully realised, the errors to which the clock is prone must be recognised and, as far as possible, be corrected for. Those which stem from the fact that the albumin clock, to the extent it exists, must be a statistical clock can be minimised by examining as many lineages as possible stemming from a particular divergence point and carrying out parallel studies with additional proteins and nucleic acids where each molecule will provide an independent estimate of the particular divergence time. We have also shown that a phylogenetic (cladistic) analysis of the immunological distance data facilitates the isolation of non-random errors stemming from discordant rates of change and immunological imperfections. We anticipate that such efforts will eventually provide molecular approaches to times of divergence with a resolving power comparable to that available from radiometric dating of objects.

We thank the following who supplied materials used: J. P. Bogart, E. D. Brodie, H. G. Cogger, G. Gentry, J. E. Juterbock, R. D. Maxson, R. C. Richmond, M. Robinson, D. L. Stephan and R. N. Wilson for frogs; R. D. Barnes, J. A. W. Kirsch, E. P. Finnie, W. Mottram, B. J. Richardson and R. B. Tesh for marsupials. We also thank Anne Hill for assistance, the National Science Foundation for financial support to V.M.S. and A.C.W., and the Alpha Helix expedition to New Guinea in 1969 for initiating the marsupial project.

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Received July 15, 1974; accepted April 15, 1975.

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Chemical communication in maternal behaviour of crayfish

I HAVE found that chemical communication is important in the brooding behaviour of the crayfish. Although chemical sex attractants have been described in the American lobster[1] and Pacific crab[4], this is the first report concerning maternal care of the young.

The female crayfish carries the eggs and subsequently the larvae on her abdominal pleopods until the third larval stage during which the larvae occasionally leave the female to feed, spending progressively less time with her. Within a few days after they moult into the fourth stage, they no longer return to the female[6]. If third stage larvae are removed from a brooding female and then placed in an aquarium containing the same brooding female and other non-brooding adults, they cluster on the former[2,3]. Non-brooding crayfish will readily feed on larvae.

These and my own observations suggested that the larvae use chemical cues to identify the brooding females, for I found that they could aggregate about the mother in the absence of visual or physical contact with her.

To investigate this further, I used ovigerous females of *Orconectes sanborni* from north-eastern Ohio, *Cambarus virilis* from central Maine and *Procambarus clarkii* from a population maintained in the laboratory. They were held in isolation in aerated unchlorinated water.

I followed two procedures to determine the larval response to the chemical cues released into the water by adults. In one method, filter paper was used to collect the chemical products released by brooding females. The paper was soaked in the tanks of brooding and non-brooding *P. clarkii* adults for at least 24 h. Three 1.3-cm squares of this treated filter paper from tanks containing both brooding and non-brooding adults were offered to between 10 and 12 third stage *P. clarkii* larvae in a 30.5-cm diameter finger bowl. Larval preference for a given adult's chemical product was indicated by the number of larvae that had aggregated on the paper squares from the tanks of different adults 10 min after the larvae were released from confinement.

Fig. 1 Two-choice maze. Larvae responded to the stimuli of the adults by moving from the main chamber into the test chamber containing water in which either a brooding female or male had been held.

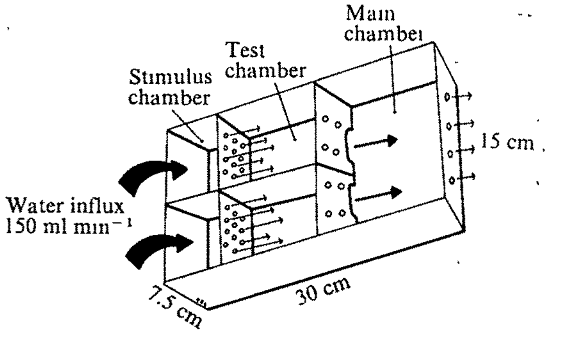

Table 1 Response of *P. clarkii* larvae to soaked filter paper

No. of larvae used	Stimulus from	No. of larvae responding	No. of experiments	χ^2 Score	*P*
242	Mother and male	152 24	24	93.09	0.005
280	Mother and female	163 33	20	85.34	0.005
180	Male and female	61 82	12	3.08	0.995

Some larvae did not respond, presumably because of lower activity levels or related motivational factors.

Table 1 shows that filter paper from *P. clarkii* brooding females was significantly preferred over those from the tanks containing males or females. The larval response to the papers of males and non-brooding females was not significant. This shows that the larvae discriminate by means of chemical factors released into the water by adults. The products released by the mother are different from those of other adults and the larvae show a significant preference for them, indicating the attractive quality of her cue.

My second approach tested the larvae's attraction when water in which brooding and non-brooding adults had been held, was presented in a two-choice maze[5]. In preparing the chemical stimuli the adults had to be held in the stimulus chambers for 45–60 min to evoke a response from the larvae (Fig. 1). After this water conditioning period the adults were removed from the maze and larvae were released. Larval preference was indicated by their movement from the main chamber, where they were released, into the test chambers, which contained the chemical stimulus of either brooding females or non-brooding adults.

To prevent the adults' stimuli from mixing in the test chambers, 200 ml of fresh water was added to the stimulus chambers at a rate of 150 ml min^{-1} every 15 min. The larvae that had moved into each test chamber were counted before and 3 min after each rinse. After three or four rinses, the adults were again placed in the maze, but this time the position of the adults in the stimulus chamber was switched, to control for other stimuli which might bias the preference of the larvae. After a 60-min

Table 2 Larval response in the maze

Species	No. of larvae used	Stimulus from	No. of larvae responding	No. of experi-ments	χ^2 Score	*P*
P. clarkii	375	Mother and male	242 51	18	124.51	0.005
O. sanborni	527	Mother and male	310 80	24	135.64	0.005
C. virilis	180	Mother and male	101 43	4	23.36	0 005

The results shown are the sum of the larval count from each experiment in which the greatest total number of larvae responded to both adult stimuli. As in Table 1, some larvae made no response water conditioning period, the adults were removed from the maze and larvae were counted as before.

Brooding females were always stripped of larvae before being used as maze stimuli, to control for possible larval stimuli, and conspecific non-brooding adults served as controls since their stimulus was not attractive.

In this manner 1-d-old third stage larvae of all three species were given a choice between the chemical stimuli of a brooding female and a male. In all experiments, the larvae of each species showed a significant preference for the brooding female (Table 2), by consistently selecting her side of the maze, even after her position in the maze had been changed.

The results of these experiments demonstrate that larval discrimination is based on a chemical stimulus released into the water by the brooding females. These cues must be important for the survival of the larvae in that they attract them to the female, thus facilitating the protective functions of the brooding interaction. Limited observation suggests that the chemical cues are species specific. They are not brood specific, however, for larvae are attracted as strongly to the other conspecific brooding females as to their own mother.

The research was supported by a grant from the US National Science Foundation. I thank A. Carlson, R. Hoy, D. Smith and C. Walcott for help.

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Non-Mendelian streptomycin-resistant tobacco mutant with altered chloroplasts and mitochondria

THE possibility of producing mutant plants of economic value by tissue culture techniques has attracted considerable interest and discussion[1-6], and was confirmed by the regeneration of plants from mutant cell lines[1]. The isolation of cytoplasmic mutants (all of those studied so far have carried Mendelian traits[1,2]) can be of practical importance; for example, male sterile ones in maize may be used for producing hybrid seeds. We report here, however, properties of a non-Mendelian, cytoplasmic mutant.

Streptomycin-resistant *Nicotiana tabacum* mutant SR1 (previously referred to as Str-r_1) was one of the first mutants isolated *in vitro* from cultured plant tissue and grown to a flowering plant[7]. A detailed study of the progeny of SR1 plants (F_1, F_2 and backcrosses) indicated that resistance is determined by a non-Mendelian, cytoplasmic factor. Ultrastructural changes of chloroplasts and mitochondria induced by streptomycin in streptomycin-sensitive (SS) cells were absent in the SR1 material when grown *in vitro* on a drug-containing medium, suggesting that mutation affected the organelles.

To test the progeny of SR1 plants, surface-sterilised seeds from selfing and crosses were germinated on water–agar and 10-d-old seedlings inoculated on to RMO medium (see ref. 7) with and without 5 and 500 μg ml^{-1} streptomycin, respectively. After 4 weeks, fresh weight of callus obtained from the seedlings was determined. Data from progeny tests are shown in Fig. 1.

SR1 seedlings from selfing, and those obtained by pollinating SR1 plants with pollen from SS plants (SR1 × SS cross), gave rise to streptomycin-resistant calli (that is, the growth of these calli was much less inhibited by streptomycin than that of the SS ones). SS and SS × SR1 seedlings were drug-sensitive when tested as calli on streptomycin medium. The behaviour of calli of seedlings from F_2 and backcrosses of F_1 to SR1 and SS was also always determined by the maternal phenotype. This indicates that streptomycin resistance was transmitted in a non-Mendelian, uniparental way, as other cytoplasmic genes of tobacco[8].

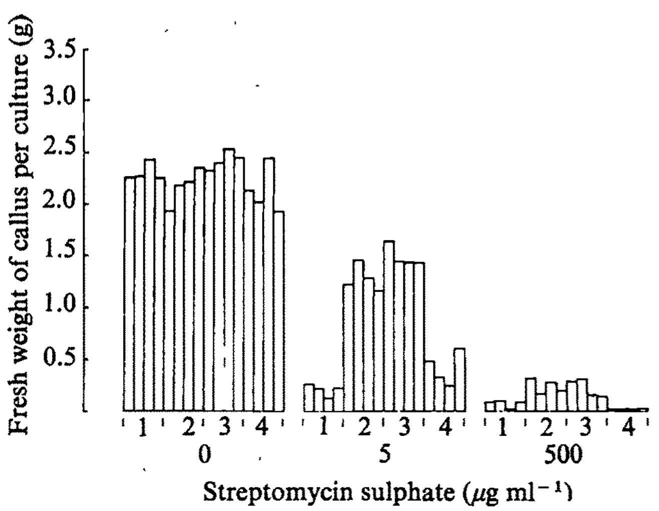

Fig. 1 Transmission of streptomycin-resistance in F_1, F_2 and backcrosses with plants regenerated from the SR1 cell line. For the test, seedlings (10 mg) were inoculated on to an RMO medium[7] (5 ml) in penicillin flasks containing different amounts of streptomycin. Seeds were obtained by crosses pollinating SS, SR1, SR1 × SS, and SS × SR1 plants (in groups from one to four, respectively) with pollen from SS, SR1, SR1 × SS, and SS × SR1 plants (columns from left to right within each group). Fresh weights (averages of ten cultures) were determined after a 4 week growth period. S.e. about 10% in each case.

Mutation in the SR1 cell line for streptomycin resistance was completely stable. So far, not one sensitive segregant was found among 100 seedlings tested from the selfed material, and no genetic change other than mutation for streptomycin resistance was observed. The diploid SR1 plants growing in a greenhouse in the absence of streptomycin could not be distinguished morphologically from the SS ones and those obtained from crosses.

In higher plants streptomycin is known to exert its toxic effect by inhibiting protein synthesis on bacterial-type ribosomes of chloroplasts and mitochondria[9]. Resistance to streptomycin of SR1 chloroplasts and mitochondria was directly confirmed by electron microscopy.

Small leaves were taken from shoots of callusing SR1 and SS seedlings, grown for 5 weeks on RMO medium, with and without 500 μg streptomycin ml^{-1}. (Shoots of SS seedlings on the drug were rudimentary.) Leaf cubes were then dissected, fixed in aldehyde solution of high osmolarity[10], treated with osmium tetroxide and embedded in araldite[11]. Thin sections of silver interference colours were viewed in a Jeol 100B electron microscope.

Ultrastructural changes of chloroplasts and mitochondria, known to occur in the SS material after streptomycin treatment[12], were found in the SS cells grown in the presence of the drug. Membranes and grana were hardly recognisable in the chloroplasts and an increase in electron density of the mitochondria was frequently found. In the SR1 cells, however, grana and stroma lamellae developed in the chloroplasts even in the presence of streptomycin. The grana were ill-defined and consisted of two to three thylakoids only, possibly as a result of heavy drug treatment. The fine structure of mitochondria seemed to remain intact.

Resistance of SR1 chloroplasts and mitochondria themselves is not the only explanation for the unimpaired ultrastructure of the organelles on a streptomycin medium. Mutation, affecting penetration of the drug into the cells or its inactivation by chemical modification (as is the case with certain bacterial

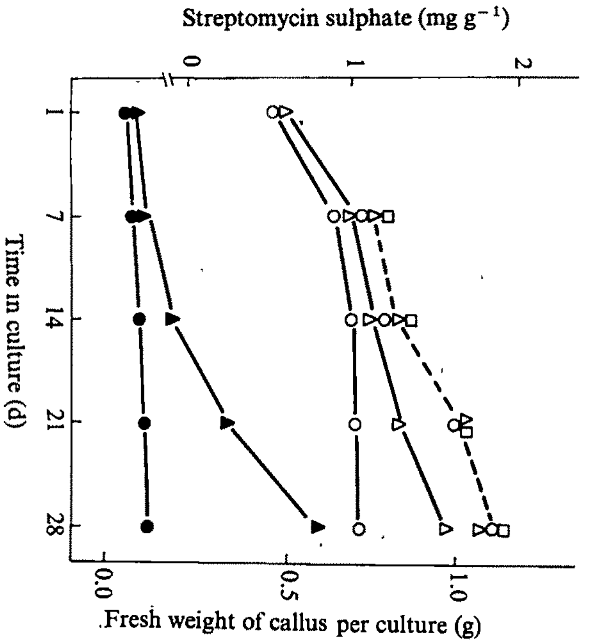

Fig. 2 Streptomycin concentration in SR1 and SS calli, and in their culture media during a 4 week growth period. Calli were grown on a medium containing 1 mg ml^{-1} streptomycin sulphate, as described in Fig. 1. ▲ and ●, average fresh weight of ten SR1 and SS callus cultures; solid line, streptomycin concentrations in the calli for SR1 (△) and SS (○); broken line, concentration of streptomycin in culture media for SR1 (△) SS (○) and uninoculated cultures (□).

strains[13]), could equally protect organelles. To exclude these latter possibilities, we studied the uptake of streptomycin in callus culture. SR1 and SS calli, grown on a streptomycin medium, were collected at weekly intervals and their fresh weights, the amount of streptomycin per unit fresh weight in calli, and culture medium, respectively, were determined (Fig. 2.).

Streptomycin was estimated in the supernatant of calli homogenised in water, and in water extracts of the culture media by the photometric method of Duda[14], except that the reaction mixture was incubated at 25 °C. The test is highly specific for streptomycin; none of the compounds present in a callus water extract or decomposition products of streptomycin, as well as other aminoglycoside antibiotics, such as neomycin, kanamycin, gentamycin, interfere (L. M., G. Kiss and E. Duda, unpublished). Agar-gel diffusion assay of the same extracts was also performed with *Bacillus subtilis* and drug concentrations were calculated from the size of inhibitory zones. As data obtained by bioassay and chemical determination were quantitatively in good agreement only one value is given in Fig. 2.

On RMO medium containing 1 mg ml^{-1} streptomycin sulphate only SR1 inocula proliferated, although there was no difference in growth in the absence of the drug (Fig. 1). Approximately the same drug concentrations were found in both SR1 and SS calli and also in their culture media, and no difference in drug content of inoculated and uninoculated media was found. Similar results were obtained when applying the drug in different amounts (not shown). Increase in drug concentration of the medium was caused by evaporation.

It is very improbable that a secondary product of streptomycin breakdown kills *B. subtilis* but still leaves the SR1 cells unharmed, because that particular derivative ought to give quantitatively the same reaction and the same activity in bioassay as streptomycin. The concentration chosen (1 mg ml^{-1}) was selected because of the convenience of quantification. When inoculating cultures with callus a much higher drug concentration was necessary to obtain difference in growth between SR1 and SS material compared with those inoculated with seedlings (Fig. 1 and Fig. 1 of ref. 7).

Uptake experiments suggest that, as in non-Mendelian streptomycin-resistant mutants of the green alga, *Chlamydomonas*[15], cytoplasmic mutation in SR1 cells affects the organelles as streptomycin was neither excluded from the SR1 cells as a result of a change in membrane permeability nor inactivated within the SR1 cells or outside in the medium.

An important inference from the present study, regarding practical applications, is that growth of cells in tissue culture under selective pressure does not necessarily induce any heritable alteration beyond the desired mutation; and, although streptomycin-resistance was obtained in cultured somatic cells, the factor responsible was transmitted by the germ cells to the progeny of the regenerated plants, and behaved as any other cytoplasmic factor detected in and described for whole tobacco plants.

We thank Drs G. Kiss and E. Duda of BRC, Szeged, for communicating their unpublished results.

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Inhibition by parasites of melanotic tumour formation in *Drosophila melanogaster*

THE cellular defence mechanisms of insects are mediated by blood cells, or haemocytes, which eliminate invading foreign organisms by phagocytosis and encapsulation. The latter involves aggregation, adhesion and flattening of haemocytes around surfaces too large to be engulfed. This process is characteristically accompanied by the intra- and extracellular deposition of melanin[1–5]. In larvae of *Drosophila* the reaction against internal metazoan parasites[6–8] is similar to the reaction which forms melanotic tumours in the body cavity of some mutant strains[9–14]. In both cases haemocytes proliferate and differentiate prematurely. In some tumorous larvae the formation of these benign, inheritable masses represents an autoimmune response in which haemocytes encapsulate abnormal cells and tissues and form compact melanised masses[11].

I report here the complete absence of the tumour phenotype when larvae of the mutant strain $vg\ tu^{28}$ are parasitised by the endophagous wasp, *Pseudeucoila bochei*. The female *P. bochei* attacks the larval stages of various species of *Drosophila*, ovipositing a single egg within the body cavity of each. When developed, the adult chews a hole through the puparium of the host and emerges. I investigated whether the initial haemocytic reactions of tumorous larvae against abnormally developing host tissues would also encapsulate the egg of the parasite, or if the normally successful parasite could not only suppress the cellular immune reaction, but also inhibit the autoimmune response associated with tumorigenesis. The only previous

Table 1 Effect of parasitisation by *P. bochei* on the incidence of tumours in *vg* tu^{28}

No. of pupae	% Tumours	% Encapsulated parasites
	Non-parasitised pupae	
118	44.91	
61	47.54	
107	50.46	
27	51.85	
88	59.09	
69	39.13	
	Mean 48.80	
	Parasitised hosts containing live parasites	
210	0.0	0.0
	Parasitised hosts containing dead parasites	
7	57.1	0.0

attempt to parasitise a tumorous insect to observe altered cellular responses was made by Walker[6] who used resistant and susceptible strains of *P. bochei*.

I examined the effects of parasitisation on tumorigenesis by parasitising *vg* tu^{28} larvae during the early second instar (72±4 h), about 24 h before tumours usually appeared. The larvae were exposed to the parasites for 2 h. Tests were conducted in a regimen of 16 h light at 23±1 °C. All hosts were dissected 2–4 d after pupation to determine the condition of the parasite and the effect of parasitisation on the cellular reactions associated with encapsulation and tumorigenesis.

In non-parasitised larvae the incidence of melanotic tumours in *vg* tu^{28} was approximately 48% (Table 1). Most larvae had one large tumour, usually in the posterior region of the body cavity. Occasionally there were four or five large pigmented masses (Fig. 2). No attempt was made to classify separately individuals with either multiple tumours or tumorous masses of different sizes. The tumours comprised fat body cells, encapsulated by haemocytes and melanised (Fig. 2). All tumours were formed before pupation, usually during late second and early third (last) instars. Tumours were retained within the body cavity throughout development and into the adult stage with no evidence of dissolution.

Hosts containing live parasites developed no melanotic tumours. Of the 217 parasitised insects dissected, seven hosts each contained a dead parasite egg. Only four of the seven hosts (57.1%) had the tumour phenotype (Table 1). There was no evidence of parasite encapsulation in any host examined. I do not know why a small percentage of eggs failed to develop, but as this is also encountered in non-tumorous host larvae, female *P. bochei* may sometimes oviposit inviable eggs. In a group of infected *vg* tu^{28} pupae that was not dissected, the parasites developed and emerged with no apparent difficulty.

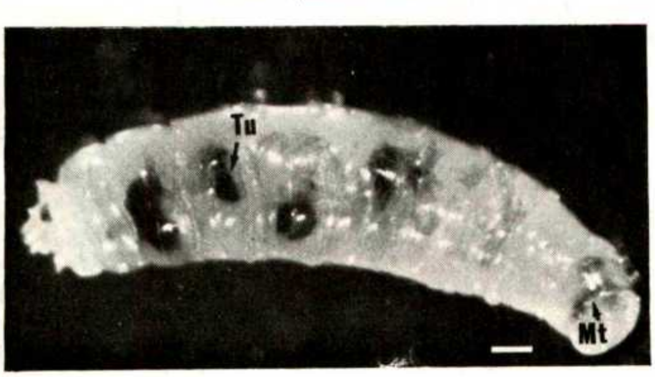

Fig. 1 Third-instar larva of a tumour strain (*vg* tu^{28}) of *D. melanogaster*. Four large melanotic tumours can be seen through the body wall of the insect. Tumour, Tu; mouth hooks, Mt. The bar represents 0.2 mm.

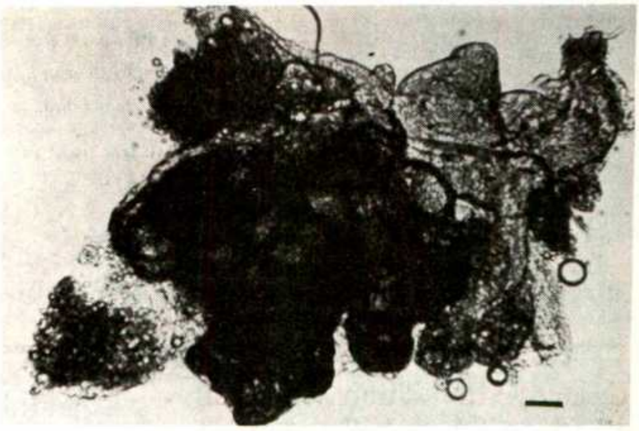

Fig. 2 Melanotic tumour dissected from the body cavity of a third-instar larva. The tumour is composed of abnormal fat body cells in the process of becoming completely melanised and encapsulated. The bar represents 0.02 mm.

It is interesting that some parasites can develop within hosts that are potentially immunologically hostile. These states of apparent immunological inactivity may represent specific failures of the immune system to recognise parasites as foreign, or they may be attributed to the ability of certain parasites to inhibit host immune responses. Such an encapsulation-inhibiting capacity has been proposed for *P. bochei*[6,7,15] and other insect parasites[16,17]. Walker's[6] studies of the cytological and genetic aspects of capsule formation in *D. melanogaster* suggest that the parasite produces a substance(s) which suppresses the proliferation and differentiation of host haemocytes. In a tumour-mutant stock (*tu* B^3) she found that the frequency of tumours was reduced from 71% to about 47% when the larvae were parasitised by *P. bochei* that were normally resistant to encapsulation, and slightly higher (80%) when infected by a parasite strain susceptible to encapsulation.

Regardless of whether the cellular mechanisms of *D. melanogaster* are activated in response to endoparasites or to the asynchronous growth and differentiation of certain host tissues, the haemocytic reactions are similar and serve to isolate and prevent the establishment of foreign or abnormal cells. The results reported here and earlier[6,7,15] suggest that the ability of *P. bochei* to suppress the encapsulation reaction of its host also inhibits a similar haemocytic response associated with tumorigenesis. Resistance of *P. bochei* in larvae of *Drosophila* may be associated with a substance elaborated from the egg of the parasite, for only in hosts containing viable *P. bochei* eggs were tumour encapsulation reactions blocked. Other factors, however, including compatible surface properties may also be involved in the resistance of *P. bochei*, for dead eggs were not encapsulated, even in hosts which formed tumours.

I thank Dr Burke Judd of the Department of Zoology and the Genetics Foundation at the University of Texas at Austin for the tumour strain. This work was supported by the Research Foundation of the State University of New York and the National Institutes of Health.

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Received March 3; revised April 10, 1975.

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Antibody stimulation of tumour growth in T-cell depleted mice

It is well known that in certain conditions the growth of allogeneic tumours can be enhanced by passively transferred antibodies directed against antigens present on the graft[1,2]. Most of the hypotheses proposed to explain this phenomenon have placed some form of antibody-mediated interference with the immune response of the host towards the tumour. Nevertheless, there have been occasional observations which suggest that immunological enhancement is the result of a change in the neoplastic cells themselves resulting from the interaction with antibody[3–5]. We have reported the results of *in vitro* experiments which support this idea[6]. Nucleoside incorporation, DNA synthesis, and cell growth were stimulated when mouse L cells were incubated in the presence of limiting amounts of rabbit anti-L cell serum (aLS); higher concentrations of antibody inhibited cell growth. Addition of complement greatly augmented cell growth, especially at low concentrations of antibody[7]. We now report that this immunostimulation phenomenon can also be observed *in vivo*. A preliminary report of this work has appeared[8].

To provide evidence that the accelerated tumour growth was not the result of an antibody-mediated alteration in the competence of the host to reject the tumour, animals with severely compromised cellular immune functions were used. The hosts were thymectomised C3H/He mice which had received 850 rad of γ-radiation and an intravenous injection of $5\text{–}10\times10^6$ syngeneic bone marrow cells one day before inoculation with tumour cells. It has been previously shown that thymectomised, irradiated, bone marrow reconstituted (Tx-B) mice have greatly impaired cellular immunity[9,10]. That this was true for the mice used in our experiments was verified by measuring the negative proliferative response of their spleen cells to the T-cell motigen, phytohaemagglutinin (PHA) according to the method of Stobo and Paul[11] (data not shown).

The tumour used in these studies was the mouse fibroblast line, L-929 (Grand Island Biological). This cell line was chosen because it has been previously shown to undergo immunostimulation *in vitro*[6]. Each animal received 2×10^6 cells suspended 0.1 ml of Eagle's MEM containing 10% heat-inactivated (56 °C, 30 min) foetal calf serum. The inoculations were made subcutaneously into previously shaved sites on the flank. The diameter of the resulting tumour was measured with calipers in two dimensions at right angles. Volume was then calculated by applying the approximation formula $V=0.4ab^2$ where a is the larger and b the smaller of the two dimensions[12].

The preparation of the aLS used in these experiments has been described previously[6]. Briefly, randomly bred New Zealand white rabbits were immunised on three occasions with 2×10^6 L cells (twice in Freund's adjuvant and once

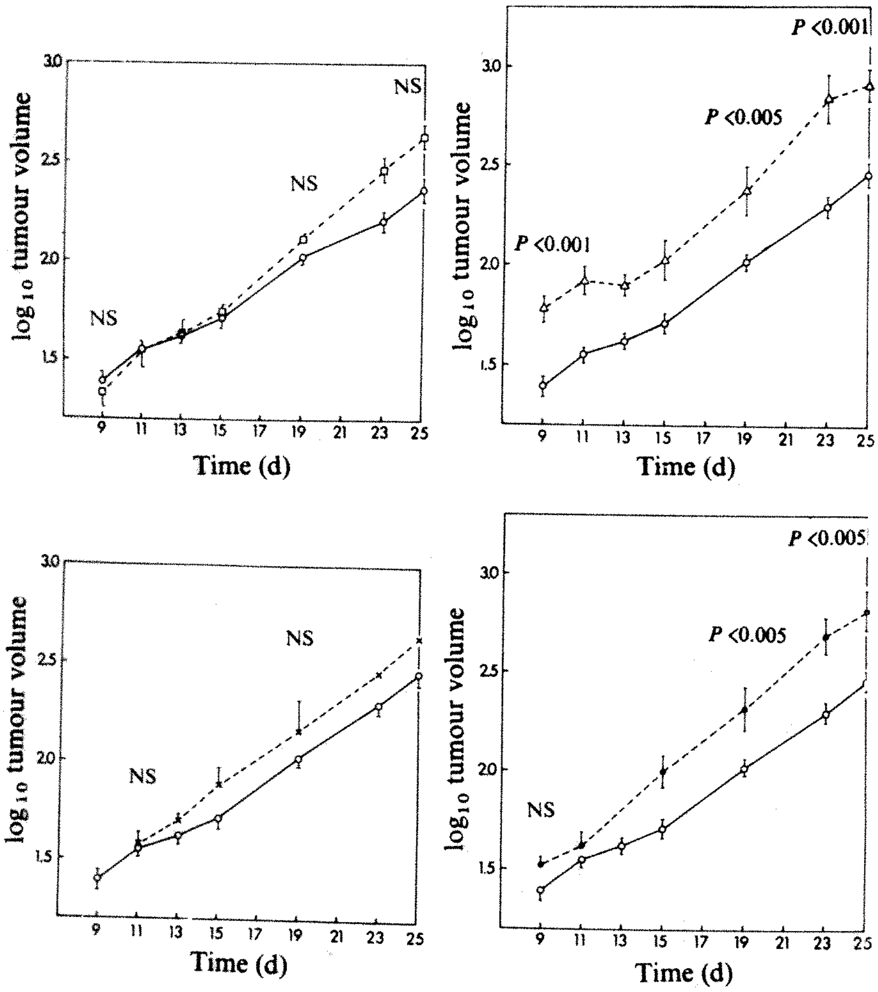

Fig. 1 Subcutaneous growth of L-929 cells in Tx-B C_3H/He mice. Previously thymectomised mice were irradiated, and reconstituted with $5\text{–}10\times10^6$ syngeneic bone marrow cells on day −1. On day 0, these animals were inoculated with 2×10^6 tumour cells. Group A ($n=17$; ○) represents pooled data from six experiments in which mice were treated with NRS. Mice in group B (three experiments; $n=7$; □) received 500 µl of aLS on day −1. Mice in group C (two experiments; $n=7$; △) received 50 µl of aLS on day −1. Mice in group D (two experiments; $n=4$; ×) received 5 µl of aLS on day −1. Mice in group E (two experiments; $n=7$; ●) received 50 µl of aLS on days −1, 5, 10, 15 and 20. Vertical bars represent s.e.m. Day 25 was chosen as the end of the observation period because significant mortality was observed beyond that time in all animals under study. NS, not significant.

in Hank's balanced salt solution). The injections were given at 2-week intervals and the serum was collected four months after the last injection. Normal rabbit serum (NRS) was prepared in the same way except that no cells were present in immunising injections. Sera were heat-inactivated (56 °C, 30 min) and absorbed for 1 h at room temperature with a crude cell suspension of C3H liver, spleen, kidney and heart (10 ml of serum per ml of packed cells).

The design of all the experiments was similar. In each instance, groups of Tx-B mice were given an intraperitoneal injection of NRS or aLS on day −1. The required amount of serum was diluted (if necessary) to 0.5 ml with phosphate buffered saline. On the following day (day 0), the mice were inoculated with tumour cells. In one experiment, additional injections of serum were given subsequent to day 0. Each separate experiment included a control group of animals which were treated identically to the experimental mice, except that NRS was substituted for aLS. Since it was found that tumour growth rate was independent of the dose or schedule of administration of NRS, data from these control mice were pooled (group A). Data obtained from mice which were treated with aLS (groups B, C, D, E) were compared with these control values, and the significance of differences determined by Student's *t* test.

The results of these experiments are depicted in Fig. 1. Animals in group B received 500 μl of aLS on day −1. This quantity of aLS produced a statistically insignificant increase in the rate of tumour growth. Animals in group C received 50 μl of aLS on day −1. Tumour growth was clearly stimulated by this quantity of antiserum (day 9, $P < 0.001$; day 19, $P < 0.005$; day 25, $P < 0.001$). Additional experiments (not shown) showed that at this dose of antiserum, immunostimulation occurred over a twofold range of cell number, that is, 1×10^6–2×10^6 cells injected were stimulated by antiserum. When the dose was decreased to 5 μl (group D), this stimulation was no longer seen. Mice in group E received 50 μl of aLS on days −1, 5, 10, 15 and 20. This treatment schedule resulted in an increased rate of tumour growth, which was again highly significant (day 9, $P > 0.05$; day 19, $P < 0.005$, day 25, $P < 0.005$.) The experiments did not attempt to answer the question of invasiveness of the tumour. The tumour grew as a solitary nodule with an occasional satellite nodule. The animals' viscera were not examined histologically for tumour metastases.

Two of our findings warrant particular discussion. First, while immunostimulation was observed when the mice were treated with 50 μl of aLS on day −1, a tenfold increase or decrease in dose abrogated the effect. Similar dose-dependent results have been described in previous studies of immunological enhancement[13,14]. Presumably, antibody is cytotoxic when present *in vivo* at high concentration (as is true *in vitro*). This cytotoxicity would nullify its ability to stimulate cells. Second, in animals given 50 μl of aLS initially, the stimulation was the same regardless of whether the dose was given only once or repeatedly. This can be explained if it is assumed that stimulation is a relatively early event in the development of the tumour. This may be so for several reasons (1) Growth *in vivo* alters the antigenic characteristics of the tumour cell membrane. This is unlikely because rabbit immunoglobulin is demonstrable by immunofluorescence in tumour sections taken from mice treated with aLS whereas no immunofluorescent staining is present in sections taken from controls treated with NSR (2) As the tumour develops, there is an increase in the number of antigenic sites and the quantity of antibody bound per cell is no longer sufficient to produce stimulation (3) Beyond a certain tumour volume, the growth rate of the tumour is limited by the rate of vascularisation and the exchange of metabolites, and stimulation can no longer be detected.

The L cell strain used in these experiments did not produce macroscopic tumours in normal C3H mice regardless of whether the mice were completely untreated or given 50 μl of NRS or aLS. In contrast, progressive growth was also observed in Tx-B mice. Along with PHA data, these results emphasise the low level of host anti-tumour immunity in the Tx-B mice. Furthermore, preliminary experiments with congenitally athymic 'nude' mice confirm the results obtained in Tx-B mice. That is, treatment on day −1 with aLS stimulated the growth of L cell tumours. Thus, it is improbable that the observed stimulation of tumour growth was caused by a blocking effect of the rabbit antibody on tumour allograft rejection. Rather, since this same aLS stimulates L cells to grow faster *in vitro* in the absence of lymphocytes or antibodies from the host animal[6] the key event *in vivo* is likely to be an alteration in the physiology of the tumour cell occurring as a direct consequence of its interaction with antibody. There have been previous reports of lymphocyte mediated immunostimulation[15–17], but to our knowledge, this is the first evidence presented for immunostimulation mediated by antibody in an *in vivo* system. These observations indicate a continuing need to consider immunostimulation as well as immunoblockade in antibody-mediated effects on tumour growth.

We thank Judith Crouch for technical assistance. This work was supported by a grant from the National Institutes of Health, a Research Scholar Award of the Cystic Fibrosis Foundation to W T.S., and by a grant from the American Cancer Society.

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Received February 17, accepted April 2, 1975.

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Killer T cells in a Burkitt's lymphoma biopsy

ONE of the *in vitro* tests available for analysis of the interaction between a tumour and its host measures the ability of isolated lymphoid cells to kill tumour cells[1]. This killing is believed to reflect cell-mediated immunity *in vivo*, which is a property of thymus-derived (T) lymphocytes. For this test lymphoid cells have been used from sites such as blood, spleen, lymph nodes and thymus. But if a cell-mediated immune response is influencing tumour growth, active lymphoid cells should be present at the site of the tumour. Indeed pathologists recognise lymphoid infiltration of tumours as a prognostic sign[2].

We have reported that patients with infectious mononucleosis (IM) have killer cells, probably T cells, in the peripheral blood, which specifically kill Epstein–Barr virus (EBV)-genome-positive cell lines derived from normal subjects as well as those derived from patients with IM or Burkitt's lymphoma (BL)[3]. The aetiological role of EBV is

Table 1 Target cell lines

Name	Origin	Characteristics
Oduor	BL[3]	B cell markers EBV genome positive
Kaplan	IM[3]	EBV genome positive
PS-B-1	Established from normal lymphocytes by *in vitro* transformation with EBV[3]	EBV genome positive
K-562	Chronic myelocytic leukaemia[11]	No B or T cell markers Very sensitive to the cytotoxic action of normal lymphocytes[10]

established in IM[4], and has been suggested in BL and nasopharyngeal carcinoma (NPC)[5]. With few exceptions BL and NPC tumour cells contain the EBV genome[5,6]. The humoral immune response, against EBV-determined antigens, in patients with IM, BL and NPC has been studied extensively[5]. We have now isolated a T-cell population from an exceptionally large BL biopsy and found that it can kill the autologous tumour cells and EBV positive cell lines.

The BL biopsy was sent to Stockholm from Kenyatta Hospital, Nairobi, and a cell suspension was prepared. This suspension was centrifuged on Ficoll–Isopaque[7] to remove debris and dead cells. We obtained approximately 3×10^8 cells. About 1% T cells were present in the suspension as estimated by sheep red blood cell (SRBC) rosette formation[8]. These were isolated by a second buoyant density centrifugation of SRBC rosettes on Ficoll–Isopaque[8]. A purity of 50% T cells with a total yield of 3×10^6 lymphocytes was achieved. Peripheral lymphocytes from a case of IM and from a normal EBV-seropositive person were used unfractionated and after removal of cells with complement receptors by buoyant density centrifugation of complement rosettes[9].

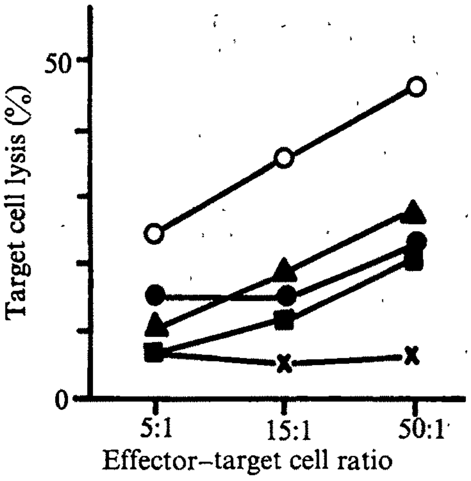


Fig. 1 Cytotoxicity of T cells isolated from the BL biopsy. Cytotoxicity tests were performed in standard V-shaped microplates in a total volume of 140 µl RPMI medium supplemented with 20% heat-inactivated foetal calf serum. 6×10^3 ^{51}Cr-labelled cells were used as targets. At the end of a 9-h incubation 100 µl of supernatant were collected. Cytotoxicity was expressed as the percentage according to the following formula: $(T-SR/M-SR)\times 100$ where T is experimental release, SR is spontaneous release of target cells in the absence of lymphocytes and M is maximal release of target cells lysed with distilled water. Characteristics and origin of target cells are given in Table 1. ○, Oduor (unfractionated 45%, fractionated −5%); ▲, PS-B-1 (unfractionated not done, fractionated 5%); ■, BL biopsy (unfractionated 15%, fractionated −6%); ×, K-562 (unfractionated 100%, fractionated 1%); ●, Kaplan (unfractionated 21%, fractionated 3%). Cytotoxicity of unfractionated and fractionated (see text) peripheral lymphocytes from a normal control, at the highest aggressor; target cell ratio, is given in brackets.

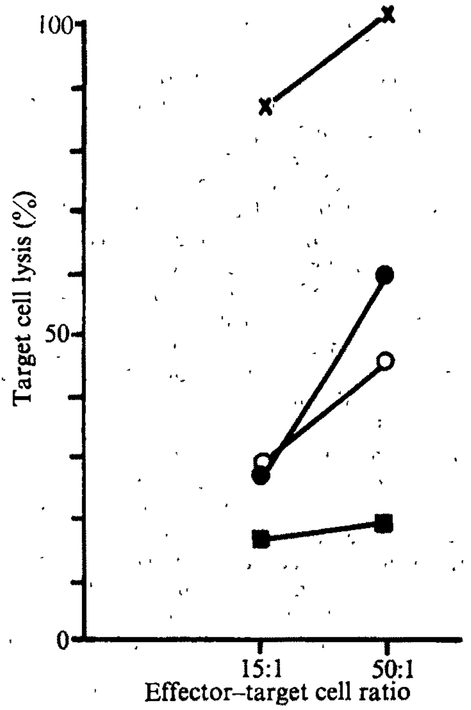
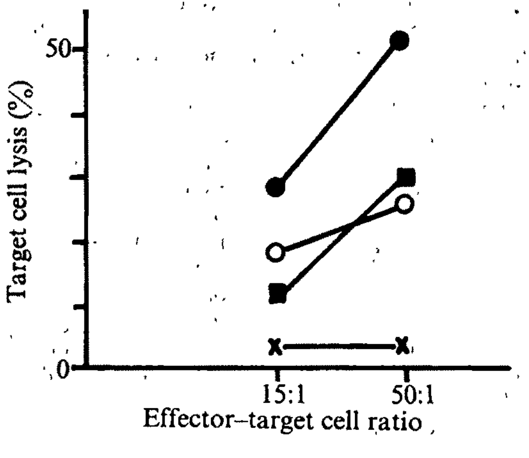


Fig. 2 Cytotoxicity of unfractionated IM lymphocytes. Symbols are the same as in Fig. 1.

T cells isolated from the biopsy killed, in a dose-dependent manner, all three EBV carrying cell lines as well as the autologous tumour biopsy cells (Fig. 1). This cytotoxicity is more impressive than it appears in Fig. 1 as the T cell suspension included 50% unlabelled biopsy tumour cells which probably reduced the indicated ratio of effector target cells by competition with the labelled target cells. A non-EBV-carrying control cell line, K-562, selected for its extreme sensitivity to killing by complement-receptor-bearing lymphocytes, present in all normal people, was hardly killed at all. The capacity of complement-receptor-bearing lymphocytes to unspecifically kill cell lines[10] is illustrated by the high reactivity of the control lymphocytes. Figures 2 and 3 further emphasise this as the nonspecific cytotoxicity against K-562 was removed with the complement-receptor-bearing lymphocytes in contrast to the specific cytotoxicity of the IM cells against the EBV-positive target cells. Furthermore, the T cells from the BL biopsy reacted most strongly against the BL-derived cell line whereas the fractionated IM lymphocytes killed the IM line preferentially. Because of a lack of IM effector cells the PS-B-1 cell line could not be included as a target cell for these cells.

Fig. 3 Cytotoxicity of fractionated IM lymphocytes. Symbols are the same as in Fig. 1.



We draw several conclusions from these experiments. First, BL patients can mount a cell-mediated, T-cell dependent, immune response against their tumours. Second, activated T cells can be recovered from the tumour site and can, in spite of their presence in a large tumour mass, still be cytotoxic against the tumour cells *in vitro*. Third, patients with IM develop killer T cells which may recognise BL tumour cells. This would be of particular interest if cell-mediated immunity could be transferred between humans. If our results hold true, BL (and NPC) patients may benefit from transfer of immunity from patients with IM.

We believe that these results, although preliminary, represent the first demonstration of specific, tumour-reactive T cells isolated from a tumour site in man. The clinical implications, for EBV-associated human diseases, remain to be elucidated.

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Platelet 5-HT uptake and release stopped rapidly by formaldehyde

UPTAKE of 5-hydroxytryptamine (5-HT) and the thrombin-induced release reaction in platelets are relatively rapid processes at 37 °C, with a linear component of approximately 1 min for uptake[1] and an estimated half life of 6 s for the release reaction[2,3]. An agent which could essentially stop these processes instantaneously and prevent any further 5-HT exchange across the plasma membrane would therefore be a valuable tool for precise studies of the kinetics of uptake and release. We describe here the treatment of platelets with formaldehyde, a technique which seems to provide such a tool.

In preliminary studies, we investigated the effect of several commonly used electron microscope fixatives on the recovery of labelled 5-HT stored in storage vesicles of human platelets Vesicular 5-HT stores were specifically labelled with ^{14}C or ^{3}H labelled 5-HT by incubating platelet-rich plasma for 30 min at 37 °C (refs 4 and 5). Platelets were then spun down, washed and resuspended in an appropriate buffer solution[3] After the various fixatives were added, the suspension was sampled for the enumeration of platelet-dense bodies[6] and the platelets then spun down for liquid scintillation counting of labelled material remaining in the platelet pellet[5].

Osmium tetroxide (OsO_4), potassium permanganate and glutaraldehyde all presented problems in the recovery of vesicle-bound 5-HT (Table 1) Recovery of label from platelets treated with OsO_4 (final concentrations of 0.01% or 0.1%) or potassium permanganate (0.1%) was very poor The fact that very few dense bodies were seen in whole mounts of platelets fixed in this fashion suggests that both agents may act as releasing agents. Even at 4 °C, where neither thrombin nor the ionophore A23187 induce release of platelet 5-HT, treatment with 0.1% OsO_4 reduced the amount of labelled 5-HT to 9% of that recovered from control pellets. This phenomenon may account for the relative infrequency of dense bodies seen in osmium-fixed platelets[7] and perhaps for the variable but generally poor visualisation of granular vesicles in adrenergic nerve terminals fixed with osmium[8]. Although glutaraldehyde (final concentration 0.25%) interfered with extraction of labelled 5-HT from pellets, it did not seem to cause loss of platelet-dense bodies.

Table 1 Recovery of labelled 5-HT after treatment with various fixative agents

	Control	Fixative agent			
		Form-aldehyde	Glutar-aldehyde	OsO_4	Potassium per-manganate
% Recovery of radioactivity, mean ± s.e.m.	100.0	102.0 ± 2.1 (n=22)	77.8 ± 3.4 (n=11)	11.4 ± 4.0 (n=34)	4.2 ± 0.3 (n=20)
No. of dense bodies per 100 platelets	625	642	612	4	6

Another fixative, formaldehyde, prepared from paraformaldehyde[9] and used at final concentrations ranging from 0.3% to 1.5%, enabled complete recovery of vesicular labelled 5-HT (Table 1). In addition, formaldehyde did not alter the trapping or binding of either ^{14}C labelled 5-HT or ^{3}H-inulin by platelets fixed before or after the addition of radioactive material and spun into pellets. About 0.6% of the inulin and a similar percentage of 5-HT (each added at concentrations ranging from 5×10^{-8} to 2×10^{-5} M) were recovered from the pellets, with or without the use of formaldehyde. Boullin and O'Brien found a similar percentage of inulin trapped in unfixed platelet pellets[10].

Formaldehyde seemed also not to affect the calcium or phosphorus content of platelet dense bodies, an important consideration since calcium and ATP may be stored[11] and are released along with 5-HT[3,12]. Electron microprobe examination[13] of dense bodies in control (unfixed) and fixed (0 3% formaldehyde) platelets revealed that the intensities of emitted X rays from both calcium and phosphorus were similar for both treated and control platelets.

Since formaldehyde permits complete recovery of vesicle-bound 5-HT, it can be used to study 5-HT remaining in platelets after various stages of thrombin-induced release. Platelets labelled with ^{3}H– or ^{14}C labelled 5-HT were resuspended and treated with thrombin[3], with formaldehyde added at appropriate times after the addition of thrombin (Fig. 1). Although we have no method to determine exactly how quickly formaldehyde stops the release reaction, the lack of release when formaldehyde was added from 1 to 5 s after thrombin suggests that it may act almost instantaneously. We also observed that formaldehyde stops release triggered by the addition of the ionophore A23187 (ref. 14). As this ionophore does not induce release by interacting with the thrombin receptor[15], it seems that the fixative is not acting simply to inactivate thrombin or the thrombin–receptor interaction. The lag before release is similar to that seen for platelet ATP as demonstrated by other methods[12].

Addition of formaldehyde also seems to be a useful method for rapidly stopping the uptake of labelled 5-HT. Labelled 5-HT was added to platelet suspensions at 37 °C, which were then treated with formaldehyde at appropriate times A final concentration of 1.5% formaldehyde was required to inhibit uptake immediately and completely. Although the conventional use of an ice bath to chill platelet aliquots to 4 °C may require up to 4 min to inhibit uptake[1] completely, one can obtain reproducible and accurate figures using formaldehyde inhibition of platelet 5-HT uptake during intervals as short as 5 s. Because of the

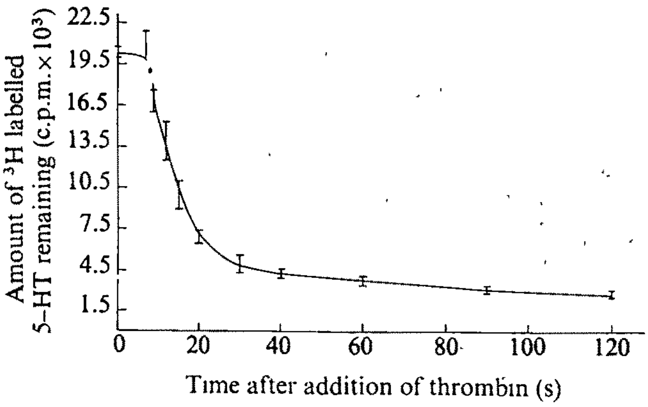

Fig. 1 A least-squares fit curve (——) for the time course of the thrombin-induced release of 5-HT from human platelets. Brackets represent the range of the actual data. Labelled platelets in suspensions at 25 °C were treated with bovine thrombin (final concentration 1 U ml^{-1}). Release was stopped by addition of formaldehyde at various time intervals after addition of thrombin. Although the curve was obtained from the data points by fitting the actual release data with an equation representing the sum of four exponentials, it is not clear that the release reaction may actually be characterised by a four-compartment model.

extremely high affinity of the platelet transport system for 5-HT, this procedure may aid in the accomplishment of more accurate kinetic studies.

Stopping either release or uptake of 5-HT with formaldehyde fixative seems to be a useful tool for studying the kinetics of these processes in detail in platelets and possibly in other suspension systems such as synaptosomes and adrenomedullary chromaffin vesicles. If proved feasible after appropriate recovery experiments, similar techniques may prove valuable with the putative neurotransmitters dopamine and noradrenaline, and possibly with acetylcholine. Further studies of electron microscope fixatives on physiological processes may also reveal interesting anomalies, such as the osmium-induced release of dense bodies observed here.

We thank Dennis Konkel and Alan Weder for technical assistance, and Karen Pettigrew and Clifford Patlak of the Theoretical Statistics and Mathematics Branch, NIMH, for help with the data on 5-HT release.

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Received March 10; revised April 8, 1975

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Rhythmic and phytochrome-regulated changes in transmembrane potential in *Samanea* pulvini

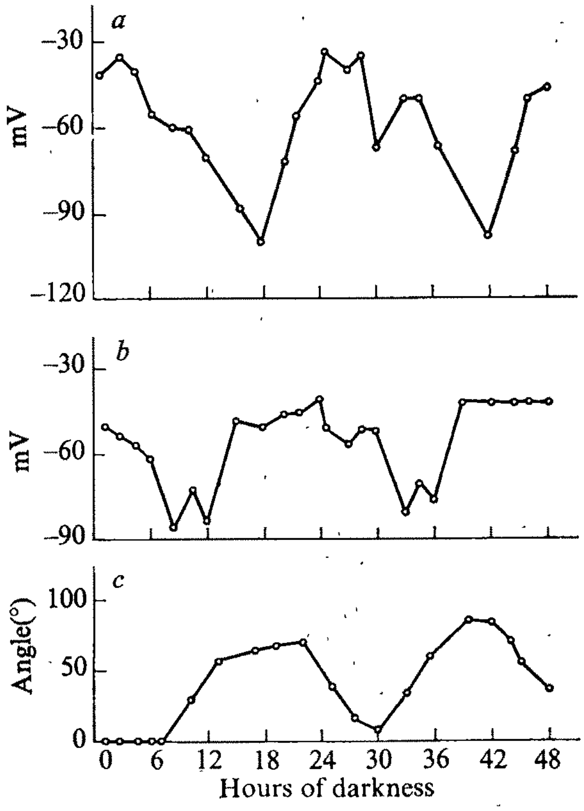
NYCTINASTIC plants such as *Albizzia julibrissin, Mimosa pudica* and *Samanea saman* have compound leaves with paired leaflets which are usually horizontal (open) during daylight and vertical (closed) at night, oscillating between these positions with a circadian rhythm during long dark periods[1–3]. The angle of a leaflet is determined by the relative turgour of motor cells on opposite sides of the pulvinus, an organ which subtends the leaflet. Extensor cells are turgid and flexor cells are flaccid when leaflets are open and vice versa when they are closed. This is regulated by movements of potassium[2,4–8], which is high in turgid cells and low in flaccid cells. Inhibitors of oxidative phosphorylation and low temperatures impede opening and promote closure, suggesting that active transport predominates during opening, while diffusion is favoured during closure[4,8–10].

Light affects K^+ movements and turgour changes through two photosystems. Prolonged, high intensity white light promotes K^+ secretion from flexor cells and leaflet opening[4,7,8]. The onset of white light acts as *zeitgeber* for rhythmic phasing when plants grown in light-dark cycles are transferred to continuous darkness[1–3]. A blue-absorbing pigment is probably the photoreceptor for these high irradiance responses[11,12]. Furthermore, brief pulses of red and far red light absorbed by phytochrome regulate nyctinastic (dark-induced) closure and also interact with the biological clock to regulate rhythmic K^+ flux and leaflet movement[2,7,8,13–16]. We now have evidence of rhythmic and light-regulated changes in transmembrane potential in motor cells of *Samanea*.

Samanea plants were grown, as before, from seed in constant humidity, constant temperature growth chambers, in cycles of 16 h cool white fluorescent light and 8 h darkness[4]. The usual dark period was extended to 48 h to study rhythmic electrical changes. A green safelight provided illumination for manipulations and measurements during the dark period. Measurements were made at 25±2 °C.

Secondary pulvini from the third to seventh uppermost leaves were cut and mounted in a Lucite chamber and perfused with a solution containing 1 mM KCl, 1 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$ and a Na phosphate buffer (1 mM, *p*H 5.6). Cells were impaled immediately after pulvini were

Fig. 1 Rhythmic changes in leaflet angle (*c*) and transmembrane potential of extensor (*b*) and flexor cells (*a*). The average s.d. was ±8%.



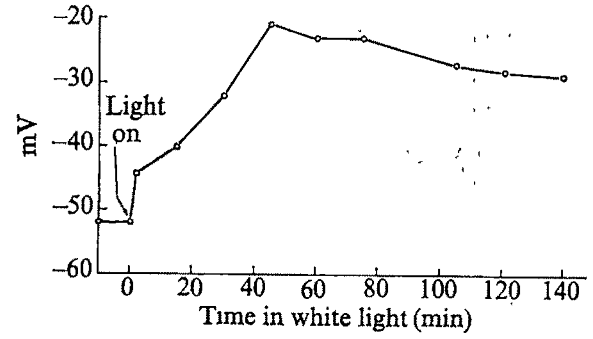


Fig. 2 Transmembrane potential of flexor motor cells of leaflets in cool white fluorescent light (about 10,800 lx). The average s.d. was ±6%.

excised, and potentials for Figs 1 and 2 were measured during the first 15–120 s after impalement. In suitable conditions, we ascertained visually the position of the microelectrode within the cell. Because of the high chlorophyll content in these cells, however, this was not always possible. In cases where the electrode was not visible, we ensured that the potential reading was indeed occurring across the membrane by taking transmembrane resistance measurements through the recording electrode. (The necessary circuitry for this procedure is integral to the WPI, M-4A electrometer and details of the theory of operation are outlined in Applications Note No. 1, April 1973. W-P Istruments, Inc. 2600 State Street, Hamden, Connecticut 06517.) Each point on our graphs is an average of four to seven cellular measurements made in three to four pulvini. Potentials that remained stable for less than 15 s were not included. Glass microelectrodes (tip diameter less than 0.5 μm and resistance 15–45 MΩ) were pulled and filled with 3 M KCl by conventional methods[21]. Each microelectrode was connected by Ag/AgCl wire to a WPI model M-4A electrometer and reference electrodes were earthed. Measurements were made on a chart recorder.

The potential of the motor cells are less negative than those reported for other plant tissues with 1 mM K^+ in the medium[22,23]. Studies with oats, however, have indicated that low negative potentials are observed in freshly cut tissue and a 24-h incubation is required to reach a more negative potential plateau[24].

Figure 1 shows changes of potential in extensor and flexor cells during 48 h of continuous darkness. Leaf angles, measured on intact plants under similar conditions of darkness, were similar to those reported previously. The relative continuity of potential measurements (data taken every 2–3 h for 48 h) provides a kinetic picture of rhythmic oscillations at the membrane. Potentials in both flexor and extensor cells oscillated with circadian periods out of phase by approximately 8 h. The rhythm in potential of the flexor cells was approximately in phase with the leaflet movement rhythm. It is interesting that the flexor cells hyperpolarised during the period described by Satter *et al.* as the energetic portion of the rhythmic cycle, and depolarised during the leaky part of the cycle[4].

Transmembrane potential in the flexor cells was also monitored during the first few hours of the experimental white light period (Fig. 2). White-irradiated leaflets open more rapidly and completely than those in the dark, presumably as a result of light-promoted K^+ extrusion from flexor cells[4]. White light caused a rapid depolarisation detectable within 1 min. During the first 45 min of the light period, the potential of the flexor cells decreased from −50 to −20 mV, and then repolarised to −30 mV.

Leaflets transferred from white light to darkness closed in about 60–90 min if preirradiated with red light (phytochrome in the P_{fr} form), but more slowly and less completely when preirradiated with far red light (phytochrome in the P_r form)[13,15]. We monitored changes in the potential of the flexor cells after the white lights were turned off and the pulvini were exposed to red or far red light. Cells were impaled before the red and far red light treatments to reveal rapid changes. Light sources for phytochrome photoconversions (red light, about 660 nm, at 350 erg cm^{-2} s^{-1} and far red light, about 730 nm, at 900 erg cm^{-2} s^{-1}) are described in ref. 25.

The potential of the flexor cells of leaflets treated with red light increased 30 mV (hyperpolarised) in 10 min with no perceptible lag (Fig. 3). Subsequent far red light reversed the red light effect within 90 s, and another exposure to red light restored the hyperpolarisation with 90 s. The potential remained high (more negative) and stable if the plants were darkened after 3 min in red light, and remained low and stable if the plants were darkened after 3 min in far red light (data not included). Thus our electrical data support the contention that phytochrome phototransformations regulate rapid, membrane-associated phenomena[26]. The lag period was shorter although the time for maximum response was longer after the first red light signal than after subsequent such signals. The first red light treatment, however, followed high irradiance white light, while the second followed far red light. Thus two photosystems (phytochrome and the high irradiance photoreceptor) interacted to regulate the first response, while only phytochrome was involved in the second. The lag after the second red light treatment was probably caused in part by the time required for conversion of appreciable P_r to P_{fr}.

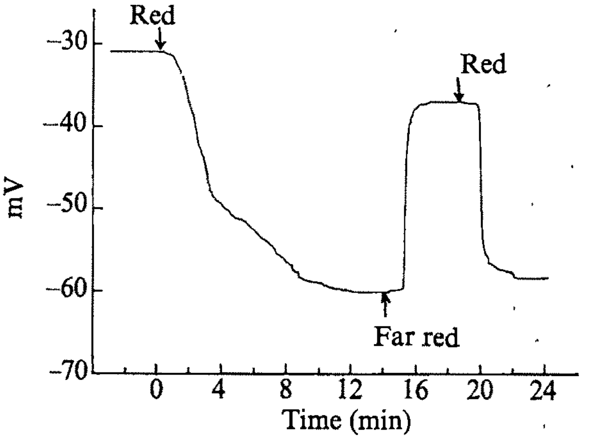


Fig. 3 Phytochrome-controlled changes in the transmembrane potential of flexor motor cells.

Kinetic studies of K^+ movement during nyctinastic closure reveal a large K^+ flux into the flexor motor cells if leaflets are preirradiated with red light, but not if they are preirradiated with far red light (our unpublished data). The K^+ flux is, however, much slower than the change in transmembrane potential, suggesting that other electrical events precede K^+ flux.

Both rhythmic and phytochrome-mediated changes in electrical parameters have been demonstrated in other systems. Spontaneous bursts of nerve action potential in the hypothalamus of the rat[17] and in the eye and parietovisceral ganglion of *Aplysia*[18] oscillate with circadian periods. Recordings from surface electrodes reveal phytochrome-regulated changes in potential of mung bean root tips and oat coleoptiles[19,20]. Ours is the first demonstration that the biological clock and two different pigment systems interact to regulate the membrane potential of the same cells. Evidence of rhythmic oscillations in electrical parameters of the membrane support the suggestion that rhythms in the composition or properties of membranes provide a basis for endogenous oscillations in various organisms[4,9,27–31]. Experiments with light stimuli provide further evidence for this view. Both white light, which entrains the leaflet movement in *Samanea*[1], and the membrane-localised chromoprotein phytochrome[32–34] which has been shown to initiate rhythms in other plants[35,36], regulate membrane electrical properties in *Samanea* pulvini.

This work was supported by grants from the US National Science Foundation to A. W. Galston and B. Etherton, both of whom we thank for reading the manuscript and making helpful suggestions.

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Received February 27, 1975.

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Inhibition of Fc receptors on a murine lymphoid cell line by cholera exotoxin

AFTER binding to its receptor, Gm1 ganglioside[1,2], cholera exotoxin has been demonstrated to be a potent activator of the adenyl cyclase system in several cell systems. The response to exotoxin is related to an increase in intracellular cyclic 3′, 5′ adenosine monophosphate (cyclic AMP). Agents that increase intracellular cyclic AMP have been shown to suppress certain immunological responses, including histamine release[3], lysosome degranulation[4], mitogenic stimulation[5], T lymphocyte rosette formation[6] and antibody-dependent cellular cytotoxicity[7]. We now report that cholera exotoxin can inhibit the expression of plasma membrane receptors for the Fc portion of IgG in a murine lymphoid cell system.

We used two cell lines derived from a spontaneous myeloma in the BALB/c mouse (PU-5). These two lines were cloned into one line bearing (Fc+) and one line lacking (Fc−) a plasma membrane receptor for the Fc portion of IgG (S. D. D. and J. Buxbaum, unpublished observation). The lines were maintained in our laboratory for 1 yr as suspension cultures at

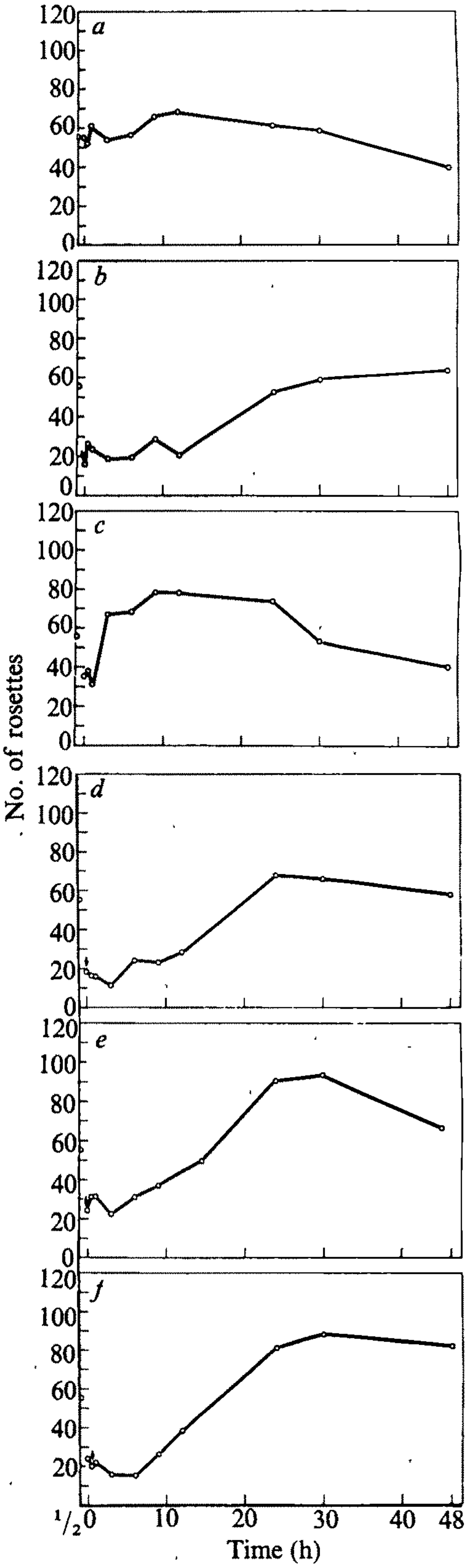

Fig. 1 Cholera exotoxin ($10\ \mu g\ ml^{-1}$), cholera toxoid ($10\ \mu g\ ml^{-1}$), aminophylline (10^{-3} M) and dibutyryl cyclic GMP (10^{-6} M) were incubated with Fc+ and Fc− cells at 2×10^{6} cells per ml in a 37 °C incubator in a 5% CO_2 atmosphere. (Arrow indicates time point of exotoxin addition.) At designated times 1×10^{6} cells per culture were removed and an EA rosette assay was performed. Viability determined was by Trypan blue dye exclusion was always greater than 70% *a*, Control; *b*, cholera toxin + aminophylline; *c*, cholera toxoid, *d*, cholera toxin, *e*, cholera toxin+cyclic GMP; *f*, toxoid+30-min pulse with toxin.

Table 1 Cholera toxin dose response of Fc+ PU-5 cell line

Cholera toxin (μg ml^{-1})	3 h Cyclic AMP (pmol per 10^6 cells)	3 h No. rosettes*	24 h Cyclic AMP (pmol per 10^6 cells)	24 h No. rosettes*
—	0.16	65	0.54	78
0.01	1.4	71	0.88	83
0.1	1.9	70	1.4	74
1.0	2.6	66	1.4	85
10.0	2.0	18	1.8	77

*Per 200 cells.
S.d. ±4.8 rosettes; the difference in rosettes between toxin and control at 3 h was statistically significant ($P<0.001$). A s.d. of ±0.28 pmol per 10^6 cells was observed in cyclic AMP assays.

37 °C in a 5% CO_2 atmosphere at a concentration of approximately 1.5×10^6 cells per ml in Dulbecco's modified essential medium containing 20% horse serum. Fc receptor activity was assayed using 0.5% sheep erythrocyte suspension coated with a 1:400 dilution of rabbit Forssman antibody (EA) and cells binding at least three erythrocytes were considered positive; at least 200 cells were counted[8]. Cholera exotoxin (Lot 0172) prepared by R. A. Finkelstein[9] under contract for the National Institute of Allergy and Infectious Diseases, and cholera toxoid (Lot 11101) prepared by treatment with glutaraldehyde[10] were obtained from C. Miller. Aminophylline and dibutyryl cyclic GMP were purchased from Sigma Chemical Co.. Cyclic AMP[11] was analysed using a radioimmunoassay kit (Schwarz–Mann) and intracellular cyclic AMP was extracted in hot sodium acetate[5]. For morphological studies, cells were fixed in glutaraldehyde, followed by 25% glycerol[12], fractured at −100 °C in a Balzer's 301 freeze etch machine, shadowed with platinum–carbon and the replicas were examined in a Siemens 102 electron microscope.

Cholera exotoxin (10 μg ml^{-1}) had a significant inhibitory effect on rosette formation (Fig. 1), which was optimal after 3 h of incubation. Fc receptor activity was reduced for 12 h, gradually recovering during the next 6–10 h. Aminophylline (10^{-3} M) potentiated the effect of toxin on Fc receptor activity and dibutyryl cyclic GMP (10^{-6} M) slightly inhibited this effect. Dibutyryl cyclic GMP (10^{-6}M) enhanced rosette activity in the absence of exotoxin. Inhibition of rosette activity by toxoid differed from that by toxin in time course and degree. Optimal inhibition with toxoid occurred after 1 h, and after 3 h inhibition could no longer be detected. The kinetics of inhibition of the Fc+ line by toxoid followed by toxin 30 min later resembled the response to toxin alone. This effect may be related to competition for the Gm1 receptor, the toxin having a greater binding affinity than toxoid. Fc− cells gave 0–3% rosettes in the presence or absence of toxin and were sensitive at 10 ng ml^{-1} to activation of their adenyl cyclase system. With Fc+ cells, although as little as 10 ng of exotoxin causes increased intracellular cyclic AMP, a concentration of 10 μg ml^{-1} was required to inhibit rosette activity (Table 1). After 24 h, intracellular cyclic AMP levels were still elevated, although the Fc receptor activity was not inhibited. Supernatants of 24-h cultures of exotoxin-treated Fc+ cells inhibited rosette formation (Table 2). Toxin-treated cells which demonstrated recovery of Fc activity were refractory to repeated exotoxin-induced inhibition of rosette activity (Table 2). Freeze fracture studies indicated an increase in intramembranous particles after incubation of either cell line with cholera exotoxin (Fig. 2*a* and *b*). Quantitative studies of this phenomenon are in progress. Our findings demonstrated that treatment of a lymphoid cell line with cholera exotoxin (10 μg ml^{-1}) inhibits Fc receptor activity, with subsequent recovery. The amount of toxin required for this effect is approximately 1,000 times that required to increase intracellular cyclic AMP. After addition of cholera toxin, intracellular cyclic AMP increases in both Fc+ and Fc− cell lines, showing that these cells are sensitive to this action of the toxin.

Table 2 Fc receptor activity after 24 h incubation with cholera toxin

	No. rosettes
Cells (toxin 24 h)	71
Cells (toxin 24 h)+toxin (second pulse)	70
Control cells+control supernatant (24 h)	87
Control cells+toxin-treated cell supernatant (24 h)	29
Control cells +toxin	26

Fc+ at 2×10^6 cells per ml were incubated for 24 h in presence (toxin treated) or absence (control) of exotoxin (10 μg ml^{-1}). Cultures were centrifuged, supernatants (10 ml) control and from toxin-treated cells were decanted and 2×10^7 control cells were resuspended in each of the two supernatants. Toxin-treated cells were resuspended in fresh media and a second pulse with toxin was introduced. All cultures were incubated for an additional 1 h at 37 °C and rosette assay was performed. S. d. ±10.3 rosettes.

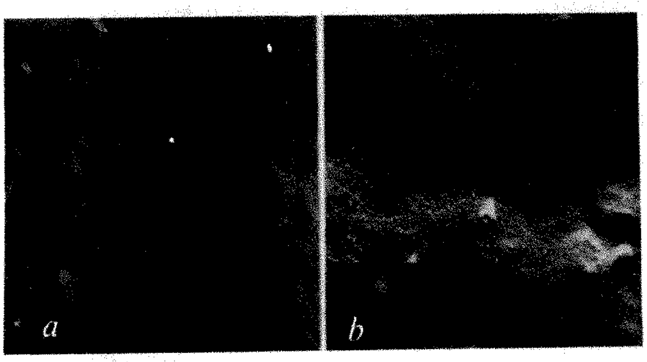

Fig. 2 Freeze fracture replicas of Fc+ PU-5 line. (×52,500) *a*, Control (3h); *b*, cholera exotoxin (10 μg ml^{-1}, 3h). Note increased number of intramembranous particles in (*b*).

Our findings indicate that cholera exotoxin inhibits Fc receptor activity, elevates intracellular cyclic AMP and may cause an increase in intramembranous particles in a murine lymphoid cell line. The potentiation of cholera exotoxin inhibition of Fc receptor activity by aminophylline and the antagonistic effect exerted by dibutyryl cyclic GMP suggests that cyclic nucleotide balance may have a dynamic role in membrane receptor expression. The dose–response relationship observed suggests that cyclic AMP levels may not directly effect Fc receptor expression, but that the inhibition of receptor activity may be associated with a secondary or tertiary effect of the cyclic nucleotides, perhaps mediated through protein kinases.

This work was supported by grants from the US Public Health Service, the National Leukemia Association, the American Lung Association and the Graduate School of the University of Minnesota.

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Received February 28; accepted April 21, 1975.

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Fc receptors on mouse placenta and yolk sac cells

MACROMOLECULAR exchange across the foeto-maternal interface in the pregnant mammal is in some species confined to the placenta, whereas in others such as the rabbit, rat and mouse, the yolk sac is also involved[1]. Whatever the route, it is well established that whereas most proteins are broken down during their passage into the foetus, a small proportion of certain immunoglobulins is transferred to the foetal circulation intact. This has important implications not only for the transmission of passive immunity but also for the access of potentially deleterious maternal antibody directed against foetal antigens.

Immunoglobulin transport into the foetus is highly selective with respect to both species of origin and class of antibody[1], and a considerable amount of discussion has centred on the possible mechanisms involved. Brambell[1,2] hypothesised that the transmission of intact antibody might depend on its attachment to cell-surface Fc receptors, which on subsequent endocytosis could afford protection from lysosomal enzyme action. Using antibody-coated red blood cells (RBC) in a rosetting technique successfully used by others to detect Fc receptors on macrophages and B lymphocytes[3,4], we have now demonstrated the presence of these receptors on both placental and yolk sac cells in the mouse.

Yolk sac membranes were dissected from mice on days 15–18 of gestation and cell suspensions prepared as described previously[5]. Contaminating erythrocytes were lysed by treatment with 0.83% ammonium chloride[6], and the preparations washed in a large volume of phosphate-buffered saline (PBS) and resuspended in medium RPMI and 10% foetal calf serum to a concentration of 1×10^6 ml^{-1}. Aliquots (0.5) ml of the suspensions were dispensed into the wells of a leukocyte migration plate (Sterilin) and incubated overnight in 5% CO_2 in air at 37 °C. Cultures were then washed by inversion in a Petri dish containing PBS, and 0.4 ml of a 2% suspension of indicator RBC added to each well. After 60 min incubation at room temperature, the cultures were washed and the cells binding RBC ('rosettes') enumerated using an inverted microscope. In five separate experiments the percentage of cells binding RBC coated with mouse alloantibody was significantly different from control cultures with non-coated indicator RBC ($P < 0.01$) (Table 1, group 1). This indicates that cultured yolk sac cells possess cell-surface receptors which, like the Fc receptors on macrophages, can bind antibody complexed with its antigen. That these receptors can also bind heterologous antibody in a similar manner was shown by the use of RBC coated with either human or rabbit antibody (Table 1, groups 2–5). The binding seems to be specific for immunoglobulin since rosetting did not occur with indicator RBC coupled to bovine serum albumin.

Table 1 Binding of antibody-coated RBC to mouse yolk sac cells

Group	Mouse strain	Antibody	RBC	Mean % rosettes* ±s.d.
1	C57BL	C57 anti-A†	Mouse A strain	22.2±8.0
	C57BL	—	Mouse A strain	1.5±1.0
2	A	Human anti-D‡	Human D	15.2±2.8
	A	—	Human D	2.8±1.3
3	C57BL	Human anti-D	Human D	20.3
	C57BL	—	Human D	1.6
4	A	Rabbit anti-mouse§	Mouse	13.9
	A	—	Mouse	1.0
5	C57BL	Rabbit anti-mouse	Mouse	35.4
	C57BL	—	Mouse	1.7

*Mean percentage rosettes = Mean (No. of rosettes/No. of endoderm cells) × 100.

†C57 anti-A serum was raised by six weekly intraperitoneal injections of A strain spleen cells into C57BL mice. Bled 6 d after last injection.

‡Human anti-D serum was from a rhesus alloimmunised individual.

§Rabbit anti-mouse serum was prepared by intramuscular injection of 5×10^7 DBA mastocytoma cells in FCA, followed by several intravenous injections of 4×10^7 DBA mastocytoma cells. Bled 10 d after the last injection.

Group 1: mean of five experiments.

Group 2: mean of three experiments.

Table 2 Effect of removal of the Fc portion of immunoglobulin on the formation of rosettes by mouse yolk sac cells

Indicator RBC	Mean % rosettes* ±s.d.
IgG-coated mouse A strain†	20.4±11.4
F(ab′)2-coated mouse A strain‡	1.3± 0.6

*Mean of three experiments.

†Prepared by DEAE chromatography of rabbit anti-mouse serum and adjusted to a protein concentration of 0.4 mg ml^{-1}.

‡Prepared by pepsin digestion of the immunoglobulin fraction of rabbit anti-mouse serum, separated on Sephadex G-150 and adjusted to a protein concentration of 0.4 mg ml^{-1}. F(ab′)2 coating of the RBC was tested by agglutination with sheep anti-rabbit immunoglobulin.

To confirm that rosette formation was dependent on Fc receptors, the test was carried out using immunoglobulin from which the Fc component had been removed. The antigen-binding F(ab′)2 fragment was prepared by pepsin digestion of the immunoglobulin fraction of rabbit anti-mouse serum[7]. Yolk sac cells incubated with indicator RBC coated with this F(ab′)2 preparation were unable to form rosettes, whereas those incubated with RBC coated with intact immunoglobulin were able to do so (Table 2).

The ability of certain lymphocyte populations to form Fc rosettes can be specifically inhibited by pretreatment with alloantisera directed against their histocompatibility antigens[4], possibly suggesting a close association, on the cell membrane, of histocompatibility antigens and Fc receptors. To determine whether this was also the case with yolk sac cells, monolayer cultures of A strain yolk sac were treated for 60 min at 37 °C with C57BL anti-A strain serum before washing and incubation with indicator cells. Alloantiserum pretreatment caused a marked inhibition of the number of cells forming rosettes compared with control cultures pretreated with normal C57BL serum. On the other hand, the rosetting ability of C57BL yolk sac cells was unaffected by pretreatment with the same antiserum (Table 3).

Table 3 Inhibition of rosette formation by treatment of yolk sac cells with alloantisera

Mouse strain	% Inhibition*		
	Experiment 1	Experiment 2	Experiment 3
A	90.4	80.9	84.9
C57BL	ND	−16.7	0.7

*% Inhibition = 100 − (% rosettes in C57 anti-A pretreated cultures/% rosettes in C57BL normal serum pretreated cultures) × 100.

Cultures pretreated with serum diluted 1 to 5 in RPMI for 60 min at 37 °C.

Rosette formation carried out using mouse A strain RBC coated with C57 anti-A serum as indicator RBC.

ND, Not determined.

In the mouse little is known of the relative contribution of the yolk sac and placental routes to immunoglobulin transmission from mother to foetus. If the placenta is important, cells bearing Fc receptors should also be detectable in this organ. This possibility was investigated by the use of cultures of whole 15–18-d placentae prepared in a manner similar to that described for the yolk sac. After washing, overnight cultures were incubated with indicator RBC as before. The results indicate that cells bearing Fc receptors are present in placental preparations and that, like those of the yolk sac, they are capable of binding both homologous and heterologous immunoglobulin (Table 4).

Since the placenta is a compound organ to which both mother and foetus contribute, it is not possible to conclude that the rosette-forming cells were foetal in origin. To investigate their identity we used the ability of alloantisera to inhibit rosette formation specifically in experiments in which the foetal and maternal components of the placental preparations were of different genotype. Placentae in which the foetal component was A strain and the maternal component C57BL, were obtained by transferring A strain blastocysts to the uteri of pseudopregnant C57BL females on day 2 *post coitum*[8] and allowing development to proceed to day 16. Cell cultures were prepared as before and pretreated with alloantisera before rosetting as described for yolk sac. As shown in Table 5, rosette formation by A strain placentae from A strain mothers is almost completely inhibited by pretreatment with alloantisera. Rosette formation by A strain placentae from C57BL mothers also shows considerable inhibition after pretreatment, indicating that many of the rosetting cells in these preparations are A strain and therefore foetal in origin. Pretreatment of C57BL placenta from C57BL mothers had no effect on rosette formation .

The Brambell hypothesis for the transfer of immunoglobulin to the foetus predicts that receptors for these proteins are present either on the cell surface or within pinocytotic vesicles. Furthermore, the preferential transfer of labelled Fc as opposed to F(ab')2 fragments to the rabbit foetus has led to the suggestion that these receptors have specificity for the Fc portion of the antibody molecule[9]. The present findings indicate that such receptors exist on the surface of certain cells in both the yolk sac and placenta of the mouse.

Table 4 Binding of antibody-coated RBC to mouse placental cells

Group	Mouse strain	Antibody	RBC	Mean rosettes per field* ±s.d.
1†	A	C57 anti-A	Mouse A strain	24.9±3.8
	A	—	Mouse A strain	0.4±0.2
2	C57BL	C57 anti-A	Mouse A strain	55.3
	C57BL	—	Mouse A strain	0.3
3	A	Human anti-D	Human D	12.6
	A	—	Human D	0.0
4	C57BL	Human anti-D	Human D	23.8
	C57BL	—	Human D	0.0

*Number of rosettes per microscope field counted. Minimum of six fields scored.

†Mean of two experiments.

Table 5 Inhibition of rosette formation by alloantisera treatment of placental cells derived from normal and transferred† mouse embryos

Mouse strain		% Inhibition*			
Foetus	Mother	Experiment 1	Experiment 2	Experiment 3	Experiment 4
A	A	96.2	87.2	86.5	97.2
C57BL	C57BL	ND	17.1	−27.4	−32.3
A†	C57BL	ND	ND	78.3	52.2

*% Inhibition=100−(No. rosettes per field in C57 anti-A pretreated cultures/No. rosettes per field in C57BL normal serum pretreated cultures) × 100.

Cultures pretreated with serum diluted 1 to 5 in RPMI for 60 min at 37 °C.

Rosette formation carried out using mouse A strain RBC coated with C57BL anti-A serum as indicator RBC.

ND, Not determined.

It is of interest to consider the precise nature of these receptor-bearing cells. On the basis of their size and morphological characteristics those in the yolk sac preparations were identified as endodermal; this was supported by two further observations. Isolated conceptuses, with their surrounding yolk sac intact, were exposed briefly to a suspension of neutral red before preparation of the membrane. This procedure labelled the pinocytotically-active outer endodermal cells in contact with the dye, and allowed their identification as rosette-forming cells in subsequent cytocentrifuged preparations. Final confirmation was obtained by the use of cultures of pure endoderm prepared by the technique described previously[5] which were also able to form rosettes. Many of the rosetting cells from the placenta were foetal in origin, as shown by the egg transfer experiments, and from their frequency and general morphology it seems reasonable to suggest that they are trophoblastic, although the involvement of cells with similar characteristics, such as macrophages, cannot be discounted. The possibility of their being endodermal elements from the crypts of Duval[11] was minimised by routine removal of this region of the placenta during preparation. Note that many cells in both foetal tissues did not form rosettes. This may be related to the regional specialisation which can be observed in the yolk sac, and to the diversity in trophoblastic cell populations in the placenta[12].

It is known that heterologous antisera can produce teratogenic effects on rat embryos *in vivo* and *in vitro* as a secondary consequence of interference with yolk sac nutritive support[13]. The specific effect of alloantisera on cell-surface function, as shown by inhibition of receptor-dependent rosette formation, suggests that maternal antibody may also have the ability to influence foetal membrane transport systems. An explanation for the suggested association between human congenital abnormalities and elevated HL-A antibody levels in pregnancy[14] could be considered in these terms.

Apart from their significance for the transfer of immunoglobulin the presence of Fc receptors on placental cells may contribute to the protection of the conceptus as an intrauterine allograft. Since antigen–antibody complexes have been implicated in the suppression of cell-mediated immune reactions[15], the possession of Fc receptors by trophoblastic elements in the placenta may allow the attachment of such complexes, thereby interfering with antigen recognition by maternal immune cells.

We thank Dr R. F. Searle for performing the egg transfers, Mr R. G. Oldroyd for help with the preparation of immunoglobulin fractions and Miss V. A. Merry for assistance. This work was supported by a grant from The Rockefeller Foundation.

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Received February 21; accepted April 22, 1975.

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New calcium-mobilising agent

DURING the past three years calcium ionophores have been increasingly used to study calcium-dependent mechanisms, for example, excitation–contraction coupling[1] and stimulus–secretion coupling[2-5]. They seem to act by increasing the cytoplasmic ionised calcium concentration, but the mechanism of action does not seem to be identical in the different tissues studied. The calcium ionophore A23187 transports calcium from the extracellular space and intracellular sources towards the cytosol[2,3] whereas another ionophore X537A (Lasalocid) seems to release calcium from intracellular binding sites only[1,5]. In addition, X537A does not seem to affect all the tissues studied so far—possibly because it does not readily penetrate cell membranes.

In the present study we have investigated the effects of Br-X537A, a derivative of Lasalocid, on hormone release from and ^{45}Ca movements in the neurohypophysis. The results show that this ionophore is much more potent than X537A. In the absence of any other stimulus the addition of Br-X537A to the incubation medium induced a 25-fold increase in hormone release (Fig. 1*a*). The ionophore can also induce a twelve-fold increase in hormone secretion at a concentration as low as 2 μg ml^{-1} (Fig. 2). No attempt was made to study the hormone release at lower ionophore concentration. This release cannot have been caused by disruption of the cell membrane and leakage of cytoplasm into the medium since the concentration of a cytoplasmic enzyme lactate dehydrogenase (LDH) appearing in the medium did not increase (Fig. 1*b*). It can be concluded that Br-X537A probably induces hormone release by exocytosis. Neurohypophysial hormone release, whether evoked by electrical stimulation[6] or a high potassium concentration[7] is associated with an uptake of calcium from the extracellular space. If the drug D600 (10^{-4} M), which is known to block calcium entry and hormone release from the neurohypophysis[8], was added to the incubation medium it did not significantly affect the secretion induced by Br-X537A. The absence of external calcium did not prevent oxytocin secretion by Br-X537A but a sixfold increase in hormone release was observed (from 2.06±0.68 to 11.61±1.36 mU).

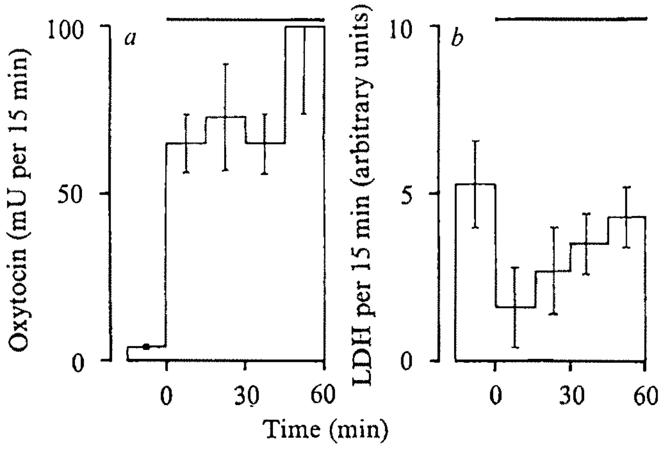

Fig. 1 Effect of Br-X537A on oxytocin release from the rat neurohypophysis (*a*); and on release of lactate dehydrogenase from glands exposed to the ionophore (*b*). After isolation of the neurohypophyses, groups of four glands were incubated for 30 min in Locke solution and then transferred to a Na-free solution for successive periods of 15 min. A Na-free solution was used to avoid dimer formation by the ionophore, which tends to occur in solutions containing sodium. Ionophore was added at a concentration of 20 μg ml^{-1} at time 0. Dimethylsulphoxide (1%) was present throughout the experiment. The oxytocin released into the incubation medium was determined by a bioassay[11] which was not affected by either dimethylsulphoxide or ionophore in the concentration used. Each column in (*a*) represents the oxytocin released during 15 min collection period (mean±s.e., $n=4$). *b*, Amount (arbitrary units) of LDH released into the medium (mean±s.e., $n=4$). This cytoplasmic enzyme was assayed by the method of Neilands[12]. Br-X537 was present during the periods indicated by the heavy horizontal bars. The composition of the Locke's solution was: NaCl, 150 mM; $CaCl_2$, 2.2 mM; $MgCl_2$, 1 mM; $KHCO_3$, 5.6 mM; glucose, 10 mM. It was equilibrated with 5% CO_2 in O_2. In the Na-free solution choline chloride (150 mM) was added to maintain the tonicity.

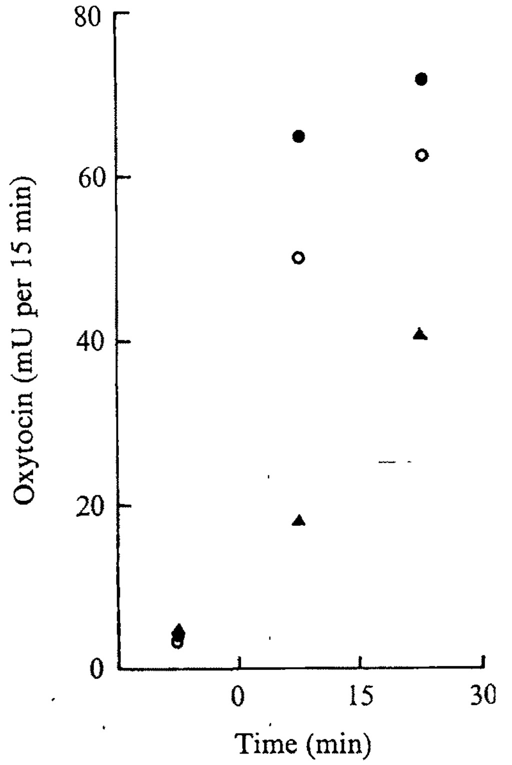

Fig. 2 Effect of ionophore concentration on oxytocin output ●, 20 μg ml^{-1}; ○, 10 μg ml^{-1}; ▲, 2 μg ml^{-1}.

These results support the suggestion that external calcium is not essential for hormone release induced by Br-X537A. Furthermore no calcium uptake was observed in the presence of the calcium ionophore in spite of an increased rate of hormone release (Fig. 3*a*). In fact, an increase in calcium efflux occurs when Br-X537A is added to the medium in which glands preloaded with calcium are incubated. Since this effect is also observed in the absence of external calcium or in a solution lacking sodium and calcium (Fig. 3*b*), the results cannot be explained by a sodium–calcium exchange previously suggested for the neurohypophysis[9,10].

Thus, although it seems that under normal physiological conditions an uptake of external calcium is associated with hormone release, the important step in the stimulation–secretion coupling mechanism seems to be an increase in cytoplasmic ionised calcium concentration. The effect of Br-X537A may therefore be to increase calcium release from an intracellular source, for example, mitochondria, as has already been suggested for the ionophore X537A (ref. 5).

Finally, this new calcium ionophore Br-X537A is very much more potent than the original molecule X537A. Although Br-X537A has not yet been shown to be a calcium ionophore in terms of physicochemical properties, it may be very useful in the study of the biological mechanisms involving calcium, particularly those in which the action of the two previous

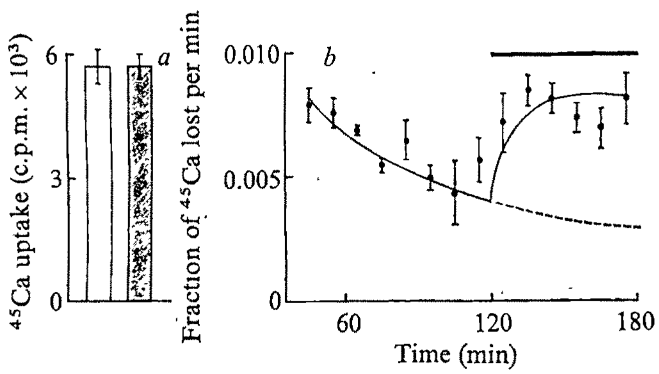

Fig. 3 Effect of Br-537A on ^{45}Ca uptake (*a*) and efflux (*b*) from the neurohypophysis. *a*, After preincubation for 30 min in normal Locke's solution both control and test group of glands were transferred for 10 min to a Na-free solution containing dimethylsulphoxide (1%). The glands were then exposed to the same solution containing radioactive calcium (7μCi ml^{-1}) for 20 min. Ionophore was present at a concentration of 20 μg ml^{-1} during the last 15 min of exposure to the label. To remove radioactive calcium from the extracellular space both test and control groups of glands were immersed for 2 s in a Na-free solution and then washed for 60 min in a similar solution which was changed every 10 min. The glands were then blotted, weighed, digested overnight in soluene and the ^{45}Ca content counted. The total calcium uptake was calculated by adding to the final ^{45}Ca gland content the amount found in the washout samples. Open column: control uptake (mean±s.e., $n=5$); hatched column: calcium uptake in the presence of Br-X537A (mean ±s.e., $n=6$). b, Groups of four glands were loaded for 60 min in a solution containing radioactive calcium (33 μCi ml^{-1}). They were then transferred to a solution lacking Na and Ca. After 10 min, the solution was removed, replaced by a fresh solution and counted. This sequence was repeated for up to 240 min. Dimethylsulphoxide (1%) was present throughout all the efflux studies. At the end of the experiment the glands were digested with soluene and the ^{45}Ca content counted. Br-X537A (20 μg ml^{-1}) was added to the perfusate as indicated by the bar. Each point represents the mean rate constant (±s.e., $n=4$) of calcium efflux observed during 180 min of washout starting at time 0. The dashed line represents the control experiments.

ionophores described, X537A and A23187, was difficult to observe.

We thank Hoffmann-LaRoche for ionophore. J.J.N. is the recipient of a European Science Exchange Programme Fellowship.

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Received February 24, accepted March 27, 1975.

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Reappearance of extrajunctional acetylcholine sensitivity in denervated rat muscle after blockage with α-bungarotoxin

IN most vertebrate skeletal muscles only the endplate region of muscle membrane is sensitive to acetylcholine (ACh), but after denervation the entire extrajunctional membrane becomes sensitive[1,2]. In rat diaphragm fibres the degree of extrajunctional ACh sensitivity increases steadily during the 7–9 d following denervation and then remains relatively constant at a high level for at least several weeks. It has been shown that this increase in ACh sensitivity results from the appearance of new ACh receptors in the extrasynaptic membrane[3,4], and an intriguing question is whether in already fully supersensitive fibres new ACh receptors continue to appear in the surface membrane. α-Bungarotoxin (α-BuTX) blocks the depolarising action of ACh on supersensitive muscle membrane[4], and is thought to bind specifically and almost irreversibly to ACh receptors. Thus one can block all or most receptors of supersensitive muscle fibres at a certain time. I report here experiments in which the reappearance of extrajunctional ACh sensitivity was followed after blocking sensitivity with α-BuTX, using denervated rat diaphragm muscle, maintained in organ culture[5].

When toxin-blocked muscles were kept at 37 °C ACh sensitivities of fibres tested during the first half hour after transfer to toxin-free medium were below 0.1 mV nC^{-1}, whereas 6 h later extrajunctional ACh sensitivity had already reappeared and was about 5% of the control value (Fig. 1). At 24 h after toxin blockage the mean ACh sensitivity had returned to 60% of the value found in unblocked control fibres of the same diaphragm, and 30–36 h after toxin block there was no statistically significant difference ($P > 0.05$) between the sensitivity of fibres exposed to toxin (317 ± 24 mV nC^{-1}; (mean ±s.e.) 40 fibres) and the sensitivity of fibres kept in normal medium throughout (346 ± 30 mV nC^{-1}; 48 fibres). Resting potentials and input resistances of control fibres and fibres exposed to α-BuTX were similar over the whole period. The time course of reappearance of extrajunctional ACh sensitivity was slower when the muscles were maintained at 27 °C, when full recovery of sensitivity to control values was found only about 70 h after toxin block (Fig. 1). Resting potentials, input

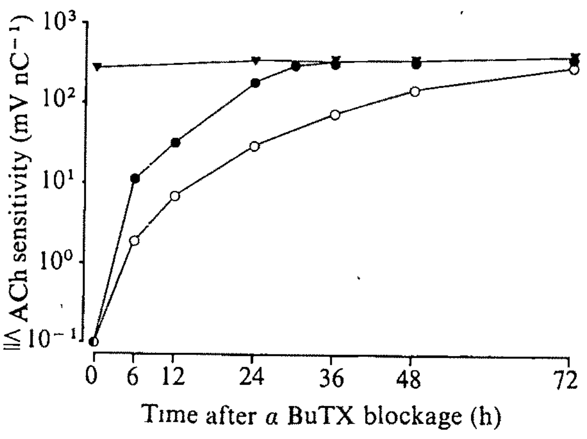

Fig. 1 Reappearance of extrajunctional ACh sensitivity after α-BuTX blockage. ●, Muscles kept at 37 °C; ○, muscles kept at 27 °C. The left hemidiaphragm of adult rats (150–200 g) was removed 9–14 d after denervation, in sterile conditions and cut into six to eight strips which were maintained in culture conditions for periods of up to 5 d (ref. 6). Muscle strips were usually kept for 24 h in normal medium before they were exposed to toxin. Blocking ACh sensitivity of all surface fibres was accomplished by adding α-BuTX (10 μg ml^{-1}) to the culture medium for 2 h. Muscle strips were then transferred to toxin-free medium to wash out unreacted toxin (zero on time scale). Subsequently the medium in the culture chambers was changed four times every half hour during the following 6 h. Thereafter changes were made at 12 h intervals. Each point represents the mean value of sensitivities found in at least 25 fibres of three different experiments. ▼, Mean sensitivities of control fibres kept in toxin-free medium throughout. Extrajunctional ACh sensitivities were determined immediately after transfer to toxin-free medium, and then at various times after toxin blockage. Sensitivity of single fibres was measured by iontophoretic application of ACh 1–4 mm from the myotendinous junction. Sensitivity measurements were made in oxygenated rat Ringer solution at 27 °C. Pulses of ACh were delivered from pipettes filled with 3 M AChCl solution, and which had resistances of 60–80 MΩ and required a bias current of 5 nA.

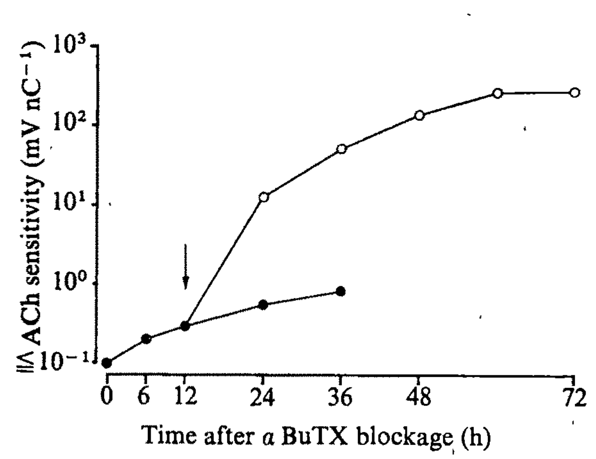

Fig. 2 Effect of cycloheximide (10 μg ml^{-1}) on reappearance of extrajunctional ACh sensitivity after α-BuTX blockage. Inhibition of resensitisation in muscles maintained in medium containing cycloheximide 12 h before, during and after toxin blockage (●). When cycloheximide is washed out by transferring muscle strips to cycloheximide-free medium (arrow) and then changing the medium at 1 h intervals for 6 h, ACh sensitivity returns (○). Muscle strips were maintained at 37 °C. Each point represents mean values from at least 25 fibres of three different experiments.

resistances and extrajunctional ACh sensitivities of unblocked fibres maintained at 27 °C were similar to those found in fibres kept at 37 °C.

The relatively fast reappearance of ACh sensitivity after block of receptors with α-BuTX may be caused by dissociation of the toxin–receptor complex. Partial recovery of ACh sensitivity in rat and mouse diaphragm has been reported previously and was attributed to a partly reversibly binding of α-BuTX[7]. Alternatively, return of sensitivity could result from the appearance of new receptors. In this case addition of cycloheximide, an inhibitor of protein synthesis[3,8,9] which is known to block the development of denervation supersensitivity[3,9,10], should affect resensitisation of toxin-blocked fibres. Therefore, the effect of cycloheximide on the reappearance of ACh sensitivity was determined. Cycloheximide (10 μg ml^{-1}) added to the culture medium abolished fibrillatory activity[6] within 5–7 h of application but had no effect on resting potential, input resistance and ACh sensitivity of unblocked muscle fibres for as long as 48 h. Yet it strongly inhibited resensitisation of toxin-blocked fibres at this concentration. For instance, 36 h after toxin block the degree of resensitisation of muscle fibres kept in medium containing cycloheximide was less than 1% of the value found for resensitisation in cycloheximide-free medium (Fig. 2). If, on the other hand, cycloheximide was washed out from the muscle 12 h after toxin blockage a delayed return of ACh sensitivity to control values was observed (Fig. 2) with a time course slower than that seen in muscles not previously exposed to cycloheximide. Similarly the time course of resensitisation was slowed when muscles were maintained before toxin blockage for 24 h in medium containing cycloheximide (10 μg ml^{-1}) which was washed out immediately after toxin blockage. For instance, sensitivity returned to a mean value of 39±4 mV nC^{-1} (40 fibres, two experiments) in these muscles 24 h after toxin block whereas in muscle fibres of the same diaphragms kept in cycloheximide-free medium before toxin blockage the corresponding value was 186±13 mV nC^{-1} (42 fibres). When muscles were maintained for only 3 h in medium containing cycloheximide before toxin blockage no effect on the time course of resensitisation was seen.

The experiments show that after block of extrajunctional ACh sensitivity with α-BuTX, there is a complete recovery of sensitivity in denervated rat muscle fibres kept in organ culture conditions. Return of sensitivity within a few hours after toxin blockage has been reported for developing chick and rat myotubes[11], and this was thought to be caused by the incorporation of newly synthesised ACh receptors into the surface membrane, as blockage of protein synthesis inhibited resensitisation in these growing fibres. The experiments also show a requirement for protein synthesis for complete resensitisation after toxin blockage in adult muscle fibres. Thus resensitisation in denervated adult muscle fibres could also reflect the appearance of new receptors in the muscle membrane. Whereas in growing myotubes the toxin–receptor complex is stable and additional receptors are incorporated into the membrane[11], in adult fibres the new receptors probably replace other receptors which are disappearing. It has recently been shown that α-BuTX, bound to rat diaphragm muscle, is lost[12,13] and this could reflect degradation of receptor–toxin complex[12].

I thank Dr J. Schmidt for providing α-bungarotoxin.

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Received March 26; accepted April 17, 1975.

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Some behavioural signs of morphine withdrawal blocked by conditional stimuli

IT has been suggested that the opiate addict gets pleasure both from the physiological effects of the drug and from the complex of environmental stimuli associated with obtaining and administering the drug. These latter stimuli achieve their control over behaviour by a conditioning process and can therefore, in theoretical terms, be considered as conditional stimuli in the Pavlovian sense. Wikler[1] proposes that the repeated occurrence of certain exteroceptive stimuli which accompany the interoceptive stimulation induced by the opiate, results in conditioning of the environment to the drug state.

This theory is receiving support from experimental studies on animals. In rats[2] and monkeys[3], it has been shown that conditional stimuli previously associated with the withdrawal of a drug can elicit physiological disturbances characteristic of withdrawal. In contrast, they may also serve to reverse withdrawal symptoms if previously associated with the injection of the drug. With-

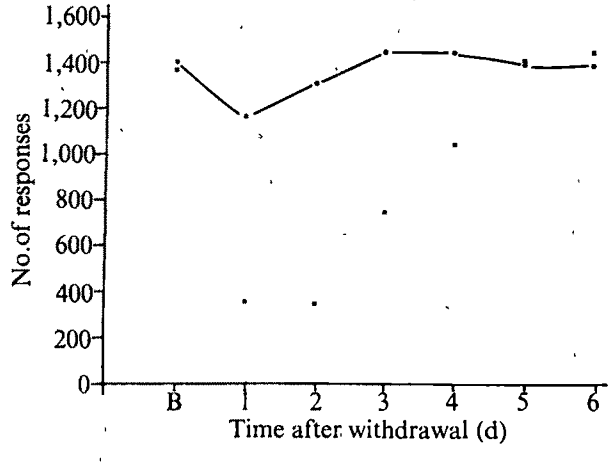

Fig. 1 Responses in VI reinforcement segment during withdrawal for tone+saline (unbroken line) and saline alone (broken line) groups. B, Number of responses on the last day of morphine injection.

Table 1 Mean (± s.e.) responses in VI reinforcement during withdrawal

		B	1	2	3	4	5	6
Natural withdrawal	Tone	1,402 (±79.5)	1,173 (±80.4)	1,315 (±63.3)	1,456 (±70.5)	1,453 (±68.2)	1,395 (±82.4)	1,397 (±82.3)
	No-tone	1,372 (±79.5)	363 (±94.1)	357 (±93.1)	753 (±92.1)	1,056 (±164.1)	1,407 (±133.1)	1,456 (±168.2)
Naloxone-precipitated withdrawal	Tone	1,482 (±65.3)	410 (±92.7)	911 (±73.6)	1,505 (±98.4)	1,449 (±126.0)	1,357 (±112.6)	1,456 (±55.6)
	No-tone	1,419 (±71.4)	296 (±77.0)	956 (±92.1)	1,359 (±64.1)	1,474 (±55.7)	1,440 (±87.4)	1,365 (±98.9)

drawal is characterised by a spectrum of unpleasant physiological effects but it also results in marked disruption of ongoing learned behaviour. We wanted to see if conditional stimuli would prevent the behavioural disruption associated with withdrawal in addition to the reported reversal of physiological symptoms[4].

Naloxone, which is thought to be a pure morphine antagonist[5], provides an experimental means of inducing withdrawal. We therefore compared the ability of conditional stimuli to reverse withdrawal symptoms induced by morphine antagonism with those of drug removal. Our results show that auditory stimuli previously paired with the morphine injection prevent the disruption of conditioned behaviour associated with morphine withdrawal but not that associated with naloxone-precipitated withdrawal.

Male Wistar rats (250–350 g) were reduced to 85% free feeding weight and then trained to press a lever for food reward (45 mg Noyes pellets) on a multiple three-component schedule using reward, time-out and a conflict schedule involving reward plus punishment. The three components were signalled by different light conditions—during reward the illumination was provided by the house light alone; time-out was signalled by darkness; and during conflict both the house light and lever lights were on. This sequence was presented twice during each testing session. Reinforcement in the reward component was available on a variable-interval 25-s (with limits of 5 and 55 s) schedule. No reinforcement was programmed during time-out. During the conflict component, reinforcement was again available on the same variable-interval schedule. Each reinforced response, however, was also punished (0.5 mA electric shock to the feet).

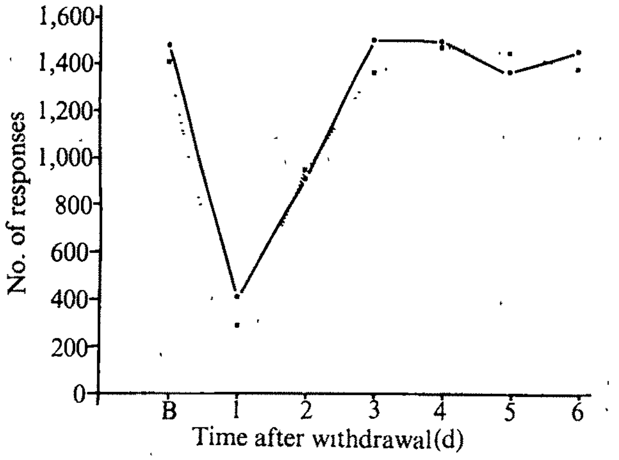

Fig. 2 Responses in VI reinforcement segment during naloxone-precipitated withdrawal for tone+saline (unbroken line) and saline alone (broken line) groups. B, Number of responses on the last day of morphine injection.

When behaviour had stabilised (the criterion was met when overall session response rates of 6 preceding days did not vary more than 10% from the overall mean for the 6 days), animals were given intraperitoneal morphine injections twice a day in increasing doses until a maintenance dose was reached. On the first day the injection was 5 mg kg^{-1} and this was increased by 5 mg kg^{-1} daily until day 8 when each injection was 40 mg kg^{-1}. Animals were then maintained at this dose, and operant behaviour was tested 30 min after the first daily injection. Injections were administered in a room next to the keeping room. Eight animals were presented with a tone stimulus (12 kHz) during the injection and for 10 min before the test session while a further eight animals received the morphine injection alone. During the second injection the tone presentation was the same but the animals were returned to the home cage after 30 min.

Initially, behaviour in all three segments of the schedule was severely disrupted by morphine. Tolerance to the rate-decreasing effects of the drug gradually developed and after 48 days of testing, animals had returned to pre-drug performance on the three segments of the schedule. Behaviour was then stabilised for a further 10 days. Morphine was then withdrawn and saline injections substituted. A two-way nested analysis of variance was performed on the data. First, considering the variable-interval reinforcement part of the schedule, behaviour was severely disrupted in the saline control group but was not significantly disrupted in animals receiving the tone stimulus plus saline. (Fig. 1 and Tables 1 and 2). Behaviour in the saline group returned to normal limits within 5 d and in both the experimental and saline groups a slight rebound effect was observed during this recovery period On day 6 of withdrawal morphine injections were resumed after testing and tolerance re-established in both groups under tone and no-tone conditions.

When behaviour had stabilised again, naloxone (0.5 mg kg^{-1} intraperitoneally) was substituted for morphine to induce withdrawal. The drug was used on day 1 of withdrawal only, after which saline injections were given. A similar analysis of variance was performed on these data. As shown in Fig. 2 and Table 1 behavioural disruption was seen in both groups indicating that conditional stimuli are unable to reverse withdrawal induced by naloxone.

Overall the data indicate that after a sufficient number of pairings with morphine injection a neutral environmental stimulus can evoke behavioural effects resembling those of morphine itself in blocking disruptive withdrawal behavioural phenomena. Responding in the extinction and

Table 2 Summary of separate analyses of variance

Factors	Natural withdrawal		Naloxone-precipitated withdrawal	
	F	d.f.	*F*	d.f.
Groups (Tone against no-tone)	16.935	1,14*	0.187	1,14(NS)
Days (days 1–6)	23.723	5,70†	50.799	5,70†
Groups × days	13.914	5,70†	0.588	5,70(NS)

NS, not significant.
**P*=0.01; †*P*=0.001.

conflict segments of the schedule was disrupted in a similar manner by morphine withdrawal. The conditional stimuli again prevented this disturbance of ongoing behaviour. Withdrawal induced by naloxone could not, however, be reversed by such conditional stimuli.

Neuropsychologically the result is interesting because it suggests that morphine activates neural pathways associated with the coding of conditional or secondary reinforcing stimuli. Recent studies[6] on the selective distribution of opiate receptors in the rat brain may provide an important clue for pursuing the neurological basis of this effect.

This work was carried out under the tenure of a SRC grant to N. C. T. On-line control of experiments used the ONLI system developed by C. Crook and S. E. G. Lea with the help of an MRC grant to A. J. Watson. We thank Professor O. L. Zangwill for extending the facilities of the Psychological Laboratory.

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Received March 12; accepted April 10, 1975.

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Antibody-bound nalorphine released on challenge with morphine

IN mice treated with morphine coupled to bovine serum albumin (BSA), antibodies are formed which bind injected morphine and reduce its analgesic effect[1]. The possible therapeutic implications of this observation have been speculated on[2], but a calculation of the number of morphine-binding ligands of a satisfactory antibody titre gives a theoretical binding capacity of only about 15 mg morphine in an adult human. The immunological removal of such low amounts of morphine would be of little practical value in the treatment of high dose intravenous abusers of opiates. One way to improve the effectiveness of the antimorphine principle is to transfer passive immunity[3]. Another way, which is described here, is the generation of an antibody-bound store of a morphine antagonist which can be released by displacement from the binding sites into an active form when morphine is injected.

In preliminary experiments female NMRI mice (body weight at end of experiment 27 ± 0.5 g) were pretreated with BSA–morphine once a week for 6 months as described by Berkowitz and Spector[1]. It was found, however, when the antiserum properties were tested *in vitro*, that to achieve a 50% displacement of ^{3}H-dihydromorphine binding, about 2×10^5 times more nalorphine was needed compared with morphine (Fig. 1a). Thus the antibody formed by BSA–morphine treatment was rather specific for morphine. For that reason a corresponding long term treatment with BSA-conjugated nalorphine was undertaken in another group of mice.

Synthesis of carboxymethyl nalorphine was carried out according to Miller *et al.*[4], by reacting β-chloroacetic acid with nalorphine hydrochloride containing a trace of ^{3}H-nalorphine. The product was purified on an anion-exchange resin (Dowex 1X4, 200–400 mesh, 13×1 cm). The carboxymethyl nalorphine formed was homogenous on paper electrophoresis at pH 6.5. Conjugation of carboxymethyl nalorphine to BSA essentially followed the method of Spector and Parker[5], except that, instead of the dialysis step, the conjugate was isolated by exclusion chromatography on a Sephadex (G-25 fine) column (30×2.5 cm). Analysis of the conjugate for radioactivity and protein showed that four or five molecules of carboxymethyl nalorphine had reacted with each BSA molecule. Each experimental animal received subcutaneously 50 μl of a solution containing Freund's adjuvant +1 μg BSA–nalorphine once a week for 6 months, and control animals were given corresponding amounts of saline or adjuvant (buffered pH 7.5) during the same time period.

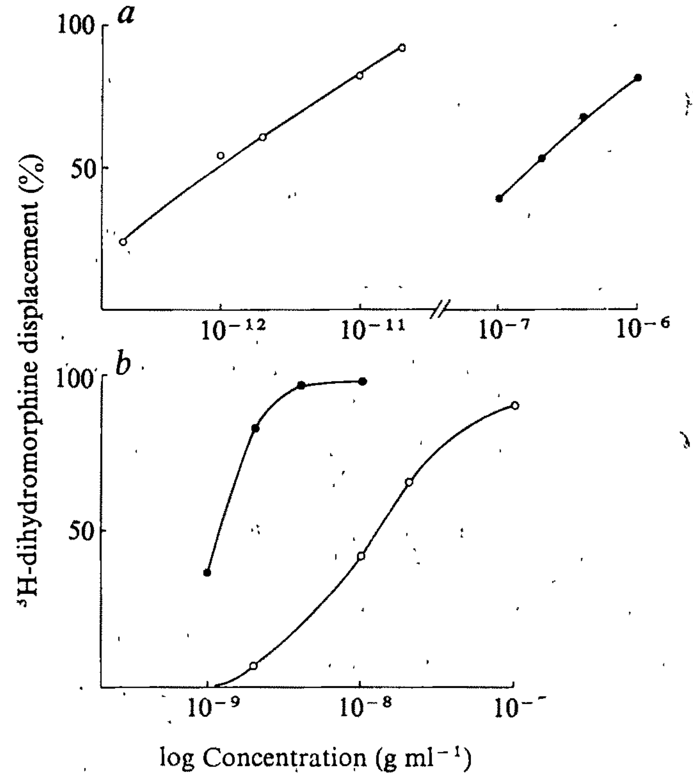

Fig. 1 *a*, Specificity of antiserum to BSA–morphine. The antiserum exhibits about 2×10^5-fold greater affinity for morphine (○) than for nalorphine (●). *b*, Specificity of antiserum to BSA–nalorphine. The antiserum had about ten times more affinity for nalorphine than for morphine.

The properties of the antiserum obtained from mice treated with BSA–nalorphine was studied in a dilution of 1 : 16 using 0.31 μCi ^{3}H-dihydromorphine (specific activity 51.3 Ci mol^{-1}; New England Nuclear) in the presence of morphine or nalorphine[5]. A 50% inhibition of ^{3}H-dihydromorphine binding to the antibodies required about ten times

Table 1 Percentage increase in pain thresholds.

Treatment once a week for 6 months	Treatment 26 h before zero time	Treatment at zero time	Percentage increase in pain threshold* (0–150 min)
Saline (n=10)	Saline	Morphine	266±25
	Nalorphine	Morphine	265±22†
Freund's adjuvant (n=10)	Saline	Morphine	229±18
	Nalorphine	Morphine	215±13†
BSA–nalorphine (n=9)	Saline	Morphine	245±18
	Nalorphine	Morphine	114±7‡

* Means ± s.e. of measurements at nine time points.
† Difference from animals receiving saline 26 h earlier not significant.
‡ Difference from animals receiving saline 26 h earlier: $P < 0.001$. Significance tested by analysis of variance, for the whole time course.

more morphine than nalorphine (Fig. 1*b*), indicating a relative specificity for nalorphine in this antiserum.

To test the *in vivo* effect on the possible immunopharmacological antagonism of morphine-induced analgesia, nociceptive stimulation was carried out[6,7]. Using a standardised electrical stimulation of the tail of the animals, the changes in the threshold for vocalisation were registered. Before administration of a drug the individual (normal) threshold was determined and after injection at regular intervals for 150 min. The graded response was expressed as percentage increase in the individual (normal) pain thresholds.

The response to morphine was tested in the three groups of mice treated chronically with saline, adjuvant or BSA–nalorphine. Twenty-six hours before the morphine injection (15 mg kg^{-1} subcutaneously), nalorphine (5 mg kg^{-1} subcutaneously) or saline was given to these three groups of animals. Table 1 shows that only in mice pretreated with BSA–nalorphine + nalorphine a significant reduction in the analgesic response occurred.

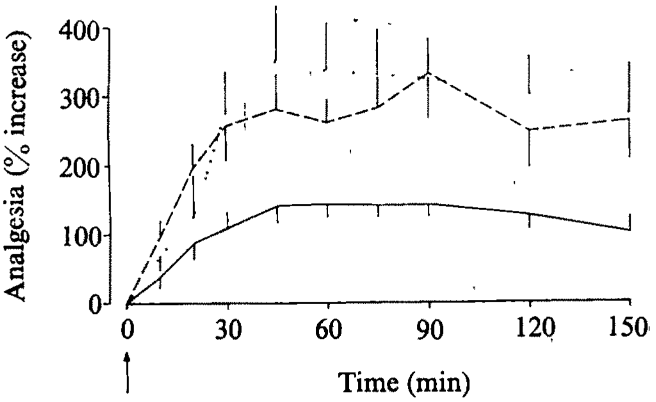

Fig. 2 Percentage increase (± s.e.) in threshold for vocalisation after saline chronically and subcutaneous administration of nalorphine (5 mg kg^{-1}) 26 h before (dotted line); BSA–nalorphine chronically and saline 26 h before (dashed line); or BSA–nalorphine chronically and nalorphine 26 h before (solid line) morphine (15 mg kg^{-1}) at arrow.

Figure 2 shows the time curves for analgesia in control mice given nalorphine 26 h before, in animals given BSA–nalorphine chronically and saline 26 h before, and in animals given BSA–nalorphine chronically and nalorphine 26 h before the morphine injection. The last combination gave a significant reduction in analgesia, indicating that the nalorphine injection 26 h before had a considerably prolonged antimorphine action. The antimorphine effect of an even higher dose of nalorphine (10 mg kg^{-1}) in morphine-dependent mice has been reported to last only 1.5–2 h (ref. 8)

Our results thus demonstrate a new immunopharmacological approach—prolonged binding of a short-acting morphine antagonist to a circulating pool of antibodies, from which it can be released by injection of morphine.

This study was supported by a grant from the Tri-centennial Fund of the Bank of Sweden and S. Thänell's Memorial Fund We thank Pharmacia Ltd for samples of BSA–morphine.

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Received March 3, accepted April 7, 1975.

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Lead-induced inhibition of brain adenyl cyclase

ACUTE lead toxicity can produce such neurological signs as ataxia, peripheral neuropathy, seizures, coma and even death[1]. Chronic low level exposure to lead, however, may be accompanied by more subtle defects in behaviour, such as hyperactivity, aggressiveness and shortened attention span[2-4]. Although lead can cause small (15–25%) and variable changes in the level of brain catecholamines[3,5,6], no biochemical basis has been clearly established for these behavioural defects. We have now found, however, that very low concentrations of lead inhibit adenyl cyclase activity in mammalian brain tissue.

The basis for our investigation is the recent evidence that the effects of certain neurotransmitters may be mediated by adenosine 3′,5′-monophosphate (cyclic AMP), which is formed in neurones through the activation of the membrane-bound enzyme, adenyl cyclase[7]. In the mammalian caudate nucleus, for example, a dopamine-sensitive adenyl cyclase has been identified with properties quite similar to those of the caudate dopamine receptor[8]. Furthermore, in the cerebellum, a noradrenaline-sensitive adenyl cyclase seems to mediate the inhibitory effect of noradrenaline on Purkinje cells[9]. Because both dopamine and noradrenaline are known to affect behaviour, we investigated the possibility that the behavioural deficits induced by toxic lead exposure may be the result of an alteration of cyclic AMP metabolism.

We examined the adenyl cyclase of rat cerebellum because the action of cyclic AMP in this tissue has been well studied both biochemically and electrophysiologically[9,10]. Figure 1 shows the effect of lead nitrate on the rate of formation of cyclic AMP from ATP in cerebellar homogenates. Enzyme activity was inhibited by concentrations of lead as low as 0.1 µM: 50% inhibition occurred at about 3 µM and almost complete inhibition at 100 µM. Lead chloride had the same effect. As well as affecting basal adenyl cyclase activity, lead ions also inhibited noradrenaline-stimulated activity. In contrast to the effect on cyclic AMP formation, similar concentrations of lead had little effect on the rate of hydrolysis of cyclic AMP, indicating that in the experimental conditions the observed decrease of cyclic AMP formation was the result of inhibition of adenyl cyclase and not of stimulation of phosphodiesterase.

The inhibitory effect of lead was found to be independent of

Table 1 Effect of washing and 2-mercaptoethanol on lead-inhibited adenyl cyclase activity in particulate fractions from rat cerebellum

	Activity (pmol per mg protein per min)
Control	90± 6
+$PbCl_2$ (100 µM)	0± 2
+2-mercaptoethanol (2 mM)	186±14
+$PbCl_2$+2-mercaptoethanol	29± 5
+$PbCl_2$*+washing	32± 2
+$PbCl_2$*+washing+2-mercaptoethanol	75± 2

Adenyl cyclase activity was determined as in Fig. 1, except that the source of enzyme was a particulate fraction obtained by centrifuging the crude homogenate at 30,000*g* for 30 min, suspending the pellet in 6 mM Tris-maleate buffer, and recentrifuging and washing twice more.

* In some cases the homogenate was preincubated with lead (100 µM) for 10 min at 30 °C before centrifugation and washing.

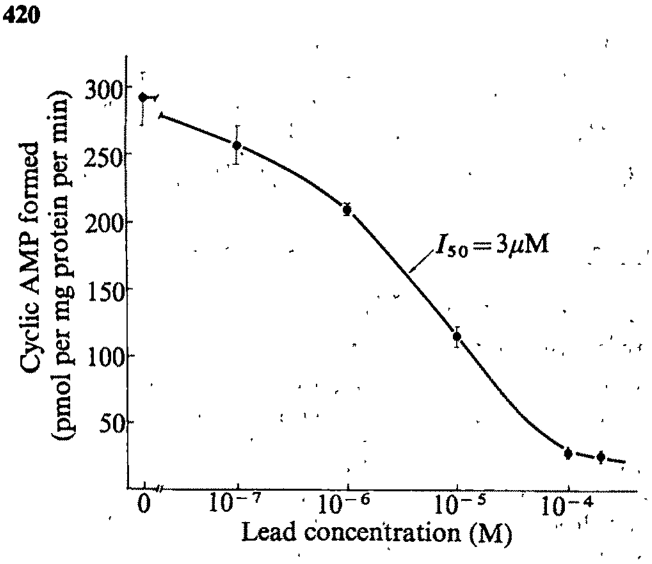

Fig. 1 Effect of various concentrations of lead nitrate on the adenyl cyclase activity of a rat cerebellar homogenate. The values shown here and in Table 1 are the means and ranges for two to four replicate samples, each assayed for cyclic AMP content in duplicate. To measure enzyme activity, 1 mm thick slices of cerebellar cortex from adult, male, Sprague–Dawley rats were homogenised (15 mg ml^{-1}) in 6 mM Tris-maleate buffer (*p*H 7.4). The rate of cyclic AMP formation was measured in an assay system containing 80 mM Tris-maleate (*p*H 7.4), 10 mM theophylline, 6 mM $MgSO_4$, 1.5 mM ATP, and tissue homogenate (1 mg wet weight) plus or minus lead salt or other test substances, in a final volume of 0.3 ml. Incubation was for 3 min at 30 °C in a shaken water bath. The reaction was initiated by addition of ATP, terminated by boiling for 2 min, and then centrifuged at low speed to remove insoluble material. Cyclic AMP in the supernatant was measured by the method of Brown, *et al.*[22]. In the experimental conditions used, enzyme activity was linear with respect to time and enzyme (protein) concentration. At concentrations of lead greater than 500 μM, the amount of cyclic AMP present in the reaction mixture increased. Much of this increase was found to be caused by a non-enzymatic conversion of ATP to cyclic AMP.

the concentration of ATP used, making it unlikely that enzyme inhibition was simply caused by a decrease in the concentration of available substrate (which might have occurred because of the known non-enzymatic hydrolysis of ATP by lead[11]). The inhibitory effect of lead was also found to be independent of the tissue protein concentration present in the reaction mixture.

In other experiments in which washed particulate fractions were used as a source of enzyme (Table 1), the inhibitory effect of lead was similar to that found in homogenates. Furthermore, tissue preparations which had first been exposed to lead ions and then extensively washed in lead-free buffer showed only a 30% recovery of enzyme activity. We also found that treatment of particulate fractions with the sulphydryl-reducing agent, 2-mercaptoethanol, increased control activity (an effect described previously[12]) but had little effect on lead-inhibited activity. Moreover, after exposure to lead, a combination of washing and treatment with 2-mercaptoethanol only partially reactivated enzyme activity, suggesting that the inhibitory effect of lead was largely irreversible in these conditions.

The concentration of lead in the brain tissue of rats exposed to a normal diet varies from 0.5 to 1.0 μM (refs 3, 13). At this level, in our study, adenyl cyclase activity was inhibited 10–20%. In cases of experimental lead intoxication in which rats display symptoms of hyperactivity, brain lead levels vary from 3.0 to 6.0 μM (ref. 3) a concentration at which, in our study, adenyl cyclase activity was inhibited 50–70%. Future studies will be required to determine whether animals made hyperactive on lead-containing diets show inhibition of adenyl cyclase activity *in vivo*.

Several reports have suggested that cyclic AMP may be involved in the facilitation of neuromuscular transmission either in normal conditions or during neuromuscular fatigue[14,15]. It is known, also, that lead ion can block neuromuscular transmission, the effect being more pronounced after long periods of stimulation[16,18]. Thus, the inhibition by lead of adenyl cyclase activity offers one possible explanation for the inhibitory effects of lead on neuromuscular transmission. It is also known that blockade of adrenergic receptors can induce reciprocal changes in catecholamine turnover[19,20]. Since inhibition of adenyl cyclase may be equivalent to receptor blockade, it is possible that the changes in catecholamine levels reported in lead-treated animals[3,5,6] could be related to the inhibitory effect of lead on adenyl cyclase *in vivo*.

Because lead influences the activity of several enzymes[21], the biochemical basis of lead toxicity in the nervous system is likely to be complex. Nevertheless, the marked inhibitory effect of low concentrations of lead on an enzyme thought to be intimately involved in synaptic transmission suggests that interference with cyclic AMP metabolism by this ion could be a factor in some of the neurological manifestations of lead toxicity.

Note added in proof: After this manuscript was accepted, LeMay and Jarett[23] reported that lead inhibits the adenyl cyclase activity of fat cell membranes and pancreatic islet homogenates in conditions used for histochemical localisation of enzyme activity. Thus, our suggestion that inhibition of adenyl cyclase may mediate some of the toxic manifestations of lead in the nervous system may also have implications for other organ systems.

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Received February 21; accepted April 21, 1975.

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In vitro conversion of a calf thymus 8S DNA polymerase to a 7.3S species

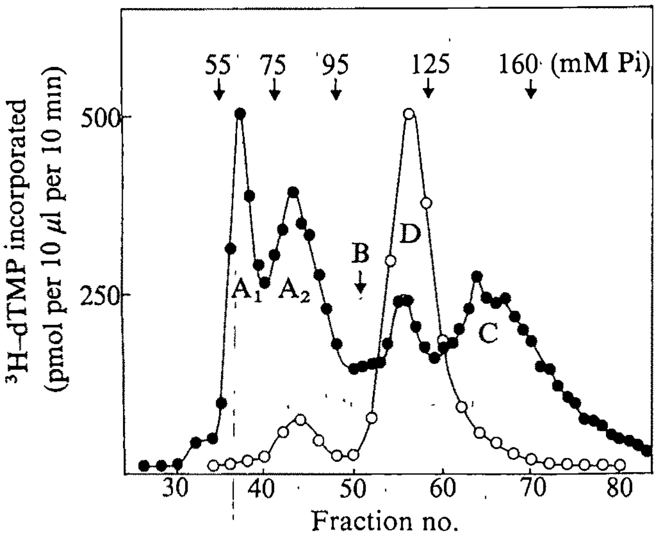
Multiple species of calf thymus high molecular weight (6–8S) DNA polymerase have been distinguished by DEAE-cellulose chromatography and partially characterised[1–4]. In order of elution these were designated enzymes A(8S), B(5.2S) and C(7.3S), and preliminary estimations indicated molecular weights of 200×10^3–230×10^3, 100×10^3–110×10^3 and 155×10^3–175×10^3 respectively[1]. Subsequent experiments have resolved the A enzyme into two components, A_1 and A_2, eluting between 55 and 75 mM and 75 and 95 mM potassium phosphate *p*H 7.8, respectively (Fig. 1 and ref. 2). The relative levels of activity of A_1, A_2, B and C vary considerably in different preparations and we show here that on urea treatment, A_2 (sedimenting at around 8S), is converted into a species sedimenting at 7.3S, the sedimentation coefficient of the C species.

A_1 and A_2 show similar properties; both elute from Sepharose 6B in 0.5 M NaCl, 0.05 M Tris HCl *p*H 8.5 (buffer I) at an elution volume (V_e) approximately 1.68 times the void volume (V_o), and both sediment on 10–30% glycerol gradients, made in buffer I, at 7.9–8.1S. When the NaCl in these gradients is reduced to 0.05 M, both A_1 and A_2 sediment with a main peak at 10.5–10.7S and a shoulder at 8.4–8.7S (ref. 2). Since the 280 nm:260 nm absorbance ratios for A_1 and A_2 were 1.6–1.7, nucleic acid is unlikely to account for the separation between them. One further feature of the DEAE-cellulose profile is the poly(dA)·oligo(dT)$_{10}$ preferring activity, designated enzyme D (ref. 1) which elutes between 105 and 125 mM potassium phosphate; it sediments on glycerol gradients made in buffer I between 6.6–7.1S. Terminal transferase and low molecular weight DNA polymerase activities were not detectable having been removed by a previous step on Sepharose 6B.

The levels of activity in peaks A_1, A_2, B and C have been found to vary in an unpredictable way in different preparations. For example in Fig. 1, the amount of 5.2S enzyme, eluting between 95 and 110 mM phosphate, is considerably reduced compared with that seen previously[1] although glycerol gradient analysis clearly reveals its presence. The proportion of enzyme C has varied between being the major and a minor component.

To explore the basis of this variability, A_1 and A_2 regions were separately pooled, concentrated and rechromatographed on DEAE-cellulose, when A_1 again eluted between 55 and 75 mM phosphate with a shoulder of A_2, and A_2 eluted mainly between 75 and 95 mM phosphate. In both cases a small amount of activity (10–20% of the total in different experiments) eluted between 125 and 160 mM phosphate in the region of enzyme C (7.3S). This was too far removed from the A enzymes to be a simple contaminant. On preincubating A_2 at 0 °C with 2.4 M urea (a concentration which caused only slight loss of enzyme activity), the activity eluting between 125 and 160 mM phosphate increased substantially (Fig. 2*a*). Recovery of activity from columns was 70–85% of that loaded. Enzyme remaining in the A position still sedimented typically at 8–8.5S; on further exposure to 2.4 M urea 50–60% of total polymerase activity again appeared in the C region on DEAE-cellulose. If preincubation with 2.4 M urea was followed by 60 min in 2.8 M urea, complete conversion of A_2 occurred. Results with A_1 were identical to those obtained with A_2.

Fig. 1 DEAE cellulose chromatography of the calf thymus high molecular weight DNA polymerase fraction. 84,000 units, 220 mg of DNA polymerase fraction IV enzyme[1] were applied to a 50×1.6 cm DEAE-cellulose column in 0.03 M potassium phosphate *p*H 7.8 and then eluted with a 1,000-ml linear gradient up to 0.25 M potassium phosphate, *p*H 7.8; fractions, 12 ml. DNA polymerase assays using either activated calf thymus DNA or poly(dA)·oligo(dT)$_{10}$ (20:1 base ratio) were carried out as described[1]. Terminal transferase was assayed using $(dA)_4$ and a Mg^{2+}-dATP assay[5]. One unit of enzyme activity is defined as the incorporation of 1 nmol ^{3}H-dTMP in 60 min. All buffers contained 20% glycerol and 1 mM dithiothreitol. Phosphate concentrations in gradient fractions were determined at 22 °C using a Radiometer Conductivity meter (type CDM 2d). ●, Activated DNA; ○, poly(dA)·oligo(dT)$_{10}$, assays.



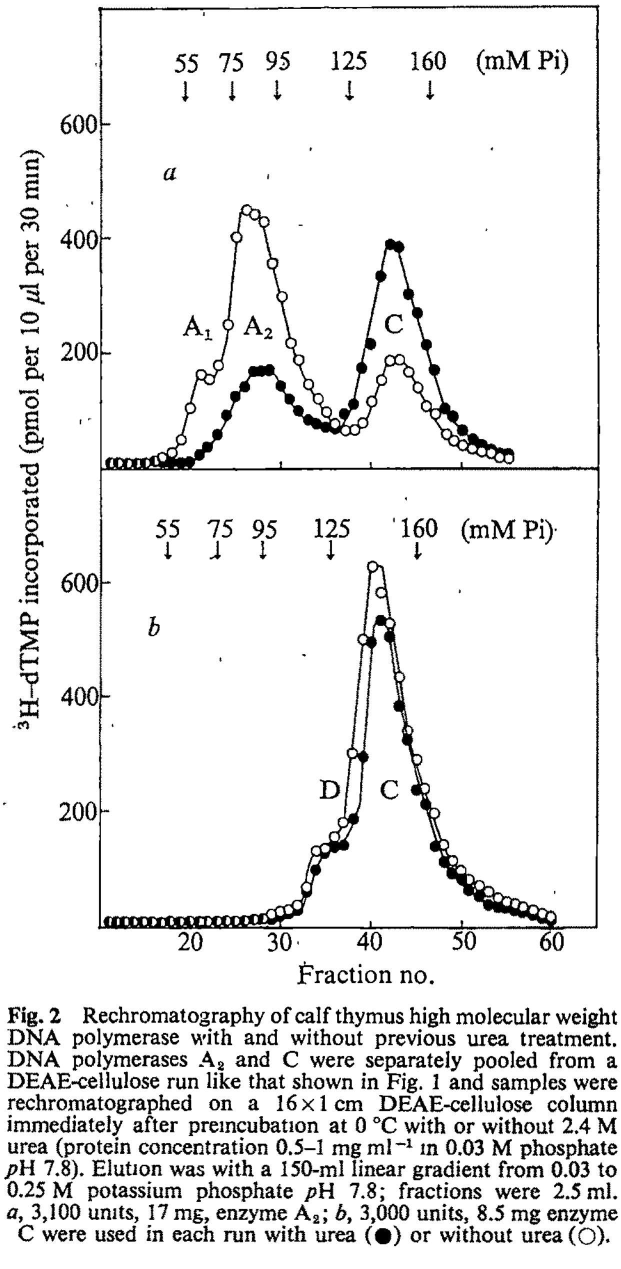


Fig. 2 Rechromatography of calf thymus high molecular weight DNA polymerase with and without previous urea treatment. DNA polymerases A_2 and C were separately pooled from a DEAE-cellulose run like that shown in Fig. 1 and samples were rechromatographed on a 16×1 cm DEAE-cellulose column immediately after preincubation at 0 °C with or without 2.4 M urea (protein concentration 0.5–1 mg ml^{-1} in 0.03 M phosphate *p*H 7.8). Elution was with a 150-ml linear gradient from 0.03 to 0.25 M potassium phosphate *p*H 7.8; fractions were 2.5 ml. *a*, 3,100 units, 17 mg, enzyme A_2; *b*, 3,000 units, 8.5 mg enzyme C were used in each run with urea (●) or without urea (○).

The enzymes derived from both A_1 and A_2 by the action of urea were pooled and found to resemble enzyme C in that they sediment on 10–30% glycerol gradients in buffer I at 7.3–7.4S (ref. 1), they elute from Sepharose 6B at V_e/V_o 1.80–1.85 (ref. 1) and they elute from DEAE-cellulose between 125 and 160 mM phosphate[1,2]. In none of our experiments has urea treatment of A or C enzymes resulted in the formation of the 5.2S enzyme (Fig. 2*b*) and although it resembles the A (8S) and C (7.3S) enzymes in several respects[1], its origin remains obscure. The elution position of enzyme D seems unaffected by 2.4–2.8 M urea treatment.

To some extent, therefore, the variability in the DEAE-cellulose elution profile can be explained by the fact that enzyme C derives from A_1 and A_2. Although the elution positions of the derived C enzymes seem to be the same on DEAE-cellulose, a slight difference in charge properties may exist and could account for the double peak in the C region of Fig. 1. In either case urea seems to remove a basic subunit of molecular weight about 50,000–70,000. Attempts to recombine C enzyme with DEAE-cellulose flow-through material, further fractionated on CM-cellulose, have not yet been successful.

This work was supported by a MRC research grant to I.R.J. and a MRC Research Studentship to I.P.H. A.M.H. is a Beit Research Fellow. We thank W. F. Wakeling for technical assistance.

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Received March 17, accepted April 22, 1975.

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More similarity between bakers' yeast L-(+)-lactate dehydrogenase and liver microsomal cytochrome b_5

BAKERS' yeast L-lactate dehydrogenase, or cytochrome b_2 (EC 1.1.2.3.), catalyses the oxidation of L-(+)-lactate to pyruvate. When purified in the presence of phenylmethanesulphonylfluoride, it is a tetramer of four presumably identical polypeptide chains of molecular weight 57,000, each of which carries one flavin mononucleotide and one protohaem IX (ref. 1). In the absence of protease inhibitor, the enzyme obtained by the classical crystallisation method of Appleby and Morton[2] shows cleavages at the N terminus and at a region about two-thirds down the chain, in each monomer; it is then composed of four chains (α) of molecular weight about 36,000 and four chains (β) of about 21,000 (ref. 1).

Yeast proteases present in the autolysate can further degrade the cleaved enzyme; the final product is a flavin-free haem-binding fragment (cytochrome b_2 core) of molecular weight 11,000[3]; trypsin digestion yields the same ultimate fragment[4]. Cytochrome b_2 core has been studied intensively as a simplified model of the haem-binding site in the flavohaemoprotein. Absorption spectroscopy[3,4], electron paramagnetic resonance[5] and nuclear magnetic resonance[6] studies, as well as photooxidation experiments[7], showed that the haem environment in cytochrome b_2 core (and thus in cytochrome b_2 itself) is very similar to that in liver microsomal cytochrome b_5. Recently completed work in this laboratory showed a marked sequence similarity between the two proteins[8]. Taken together, all these data suggest that cytochrome b_2 core has an overall three-dimensional folding very similar to that of cytochrome b_5, and that the two proteins are evolutionarily related[8].

The above findings have raised the question of the integration of the 11,000-dalton fragment in the molecular edifice of native cytochrome b_2. We carried out automatic degradation experiments on the N terminus of the intact cytochrome b_2 core subunit, and on the α chain of the cleaved enzyme. The results (Table 1) clearly indicate that cytochrome b_2 core sequence begins exactly at the N terminus of the latter, and that the yeast proteases remove the seven preceding residues when they cleave the intact enzyme during purification in the absence of inhibitor. It had already been shown that the core is part of the α chain[9,10]; our data enable a more precise determination of its location in the linear sequence, and extend the scheme presented in a previous paper[1] (Fig. 1).

It has been suggested before that the cytochrome b_2 monomer can be folded into at least two globular domains, encompassing the haem and flavin-binding sites, respectively (they could correspond to the α and β subunits); these domains may have become covalently bonded as a result of a gene fusion process[1,11]. It is clear that the cytochrome b_2 core region can constitute a structural domain in itself (ref.

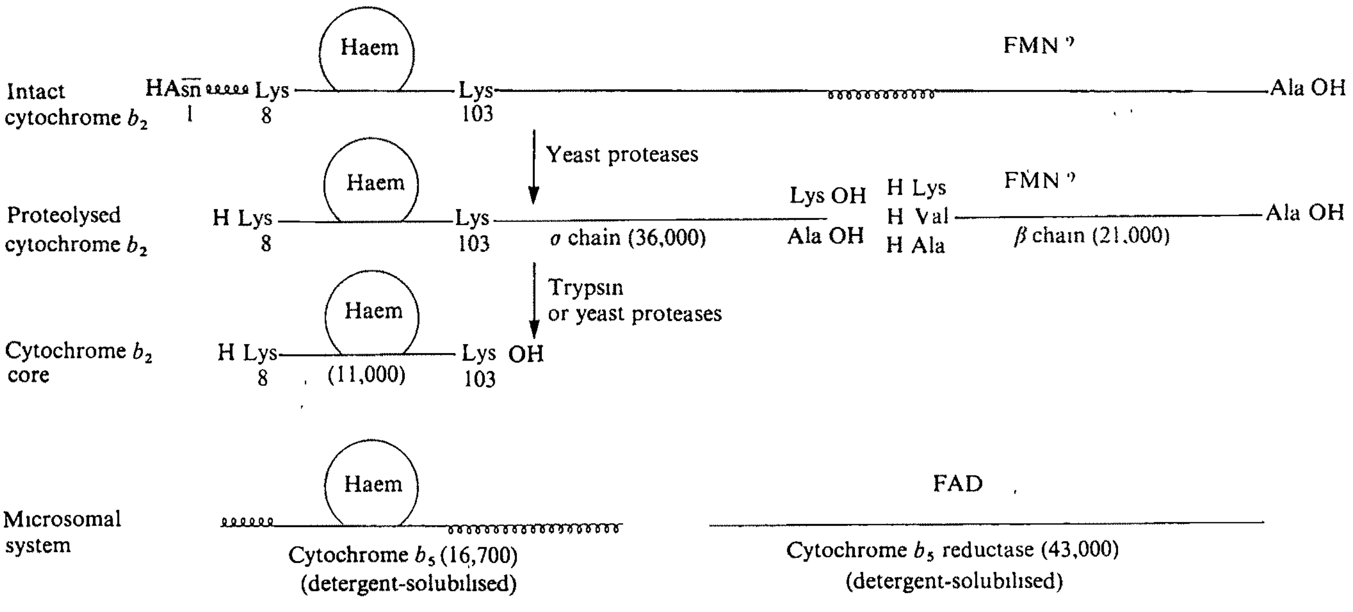

Fig. 1 Linear representation of cytochrome b_2 and cytochrome b_5–cytochrome b_5 reductase system. Spirals, fragments released by proteases. Cytochrome b_5 reductase solubilised by lysomal action has a molecular weight of about 32,000, the localisation of the lost hydrophobic fragment (at the N or C terminus) is undefined at present[21,22] FMN, Flavin mononucleotide; FAD, Flavin adenine dinucleotide.

Table 1 N-terminal sequences of various cytochrome b_2 derivatives and detergent-solubilised cytochrome b_5

Intact b_2*	H-Asn-Pro-Lys-Leu-Asp-Met-Asn-↓Lys-Gln-Lys-Ile-Ser-Pro-Ala- 1 5 10
α chain*	H-Lys-Gln-Lys-Ile-Ser-Pro-Ala-Glu-Val-Ala-Lys-His-Asn- 10 15 20
b_2 core†	H-Lys-Gln-Lys-Ile-Ser-Pro-Ala-Glu-Val-Ala-Lys-His-Asn-Lys-Pro-... 10 15 20
Human b_5‡	Z(Asx,Glx)-Glu-Glu-Ala-↓Ser-Asp-Glu-Ala-Val-Lys-Tyr-Tyr-Thr-Leu-Glu-Glu-Ile-Glu-Lys-His-Asn-His-Ser-...
Bovine b_5‡	Z(Asx,Glx,Glx,Glx,Ala,Ser)-↓Ser-Lys-Ala-Val-Lys-Tyr-Tyr-Thr-Leu-Glu-Gln-Ile-Glu-Lys-His-Asn-Asn-Ser-...
Pig b_5‡	Z-Glx-Glx-Asp-Ala-Ser-Lys-↓Ala-Val-Lys-Tyr-Tyr-Thr-Leu-Gln-Glu-Ile-Glx-Lys-His-Asn-Asn-Ser-...

The vertical arrows represent the points of specific proteolytic cleavage during preparation of the proteins. *Z*, absence of free terminal amino group.

*Determined by automated Edman degradation with a Socosi sequenator PS100, according to published procedures[8], using 0.08 µmol untreated enzyme and 0.13 µmol reduced S-carboxymethylated α chain. The former was purified according to ref. 1, the latter according to ref. 18.

†Ref. 8 and Guiard and Lederer (unpublished).

‡The sequences before and after the arrows are reproduced from Ozols[12,19] and from ref. 20, respectively.

8 and B. G. and F. L., unpublished), although we do not know how independent a structural unit it represents in the intact molecule. The fact that it is situated at the N terminus of the protein makes one wonder about its possible importance in the folding of the peptide chain during biosynthesis.

Of particular interest is the comparison with results obtained by Ozols[12] on liver cytochrome b_5 from various species. Solubilisation of that protein with detergents leads to a longer molecule than solubilisation with proteases[13,14]. In addition to a hydrophobic tail on the C terminal side, which is responsible for the attachment to the membrane[15,16], detergent b_5 has a few more amino acids on the N terminal side[12]. The partial sequences are shown in Table 1 in such a way as to respect the alignment presented elsewhere[8] between cytochrome b_2 core and proteolysed cytochrome b_5. It becomes clear (see also Fig. 1) that the structural similarity between the two fragments[8] extends to the N terminal regions of the undigested systems. These may be rather flexible, as even the first two residues of lipase-solubilised calf liver cytochrome b_5 are disordered in the crystal[17]. This flexibility could explain the easy cleavage by proteases.

The experiments reported here, in our view, strengthen the idea that cytochrome b_2 core and cytochrome b_5 may have diverged from a common ancestral protein. Important questions to be answered are how has the counterpart of the cytochrome b_5 hydrophobic tail evolved in cytochrome b_2, and whether the flavin-binding region in the yeast enzyme is homologous to cytochrome b_5 reductase (see Fig. 1).

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Received March 24; accepted April 15, 1975.

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Molecular conservation of 74 amino acid sequence of ubiquitin between cattle and man

UBIQUITIN (originally 'ubiquitous immunopoietic polypeptide' or 'UBIP')[1] induces lymphocyte differentiation and was first found in bovine thymus during isolation of the thymic polypeptide hormone thymopoietin (originally 'thymin')[2] which induces T-cell differentiation[3] and has secondary effects on neuromuscular transmission[2]. Ubiquitin, which has a molecular weight of 8,451, is interesting for several reasons. First, it induces differentiation of T cells and, unlike thymopoietin, B cells[1]. Second, both properties are inhibited by the β-adrenergic blocking agent propranolol, and so the capacity of ubiquitin to induce lymphocyte differentiation seems to depend on reaction with a β-adrenergic receptor. Third, ubiquitin activates adenyl cyclase in lymphocyte precursors and in other tissues, again through a β-adrenergic receptor (M. Bitensky, and G. G., unpublished). Finally, ubiquitin, as the name implies, is found not only in thymus but in all other animal cells and in yeasts, bacteria and higher plants[1]. Although its physiological function remains unknown, ubiquitin must be vital to the living cell to have been conserved over such a long evolutionary time span. We have reported the complete primary structure of bovine ubiquitin[4]—a single polypeptide chain of 74 amino acids—and now report that human ubiquitin has an identical sequence.

Human ubiquitin was isolated from thymus obtained from cases of sudden death in young people, the purification procedures being as described for bovine ubiquitin[1] except that the thymus was initially homogenised at a lower concentration (5% wet weight per volume). The product was pure by polyacrylamide gel disc electrophoresis[1] and end-group analysis.

Residues 1–56 were identified by automated Edman degradations of 6 mg using a Beckman model 890 sequencer as described previously[4]. The average repetitive yield was 92%. Human ubiquitin was maleated to blockade the lysine residues and cleaved with trypsin at the arginine residues as for bovine ubiquitin[1]. The four resulting peptides were resolved by molecular sieve chromatography on BioGel P-6 in a pattern identical to that obtained with bovine ubiquitin[1]; they were designated MT-1, MT-2, MT-3 and MT-4 in order of decreasing size. The amino acid content and N-terminal analyses of intact human ubiquitin and the tryptic peptides of maleated human ubiquitin (Table 1) are identical to those of bovine ubiquitin[4]. Peptide MT-1, consisting of 42 amino acids and with a blocked N-terminal residue (maleated) corresponds to the N-terminal portion of ubiquitin. Peptides MT-2, MT-3 and MT-4 were fully sequenced by manual Edman degradations with positive identifications at each position. The automated sequence determination of human ubiquitin provided the sequence corresponding to MT-1 and MT-3 and the overlap provided an order of MT-1, MT-3 and MT-2. MT-4 was placed at the C-terminus by exclusion and by homology with bovine ubiquitin. The complete amino acid sequence of human ubiquitin is summarised in Fig. 1.

The evolutionary rate of change for amino acid sequences differs greatly among various proteins[5]. The slowest-changing sequence yet recorded is histone IV with only two substitutions in 102 residues between cattle and pea[6]. Cytochrome *c*, the change of sequence of which is considered relatively slow, has 10 substitutions in 112 residues between cattle and man[5]. In comparison, the identity of bovine and human ubiquitin sequences is remarkable. Since many substitutions entail only minimal structural change one can only wonder what structural stringencies imposed on ubiquitin within the living organism are so vital as to require full conservation of a 74 amino acid sequence within these two orders of mammals.

Table 1 Amino acid composition and N-terminal analysis of human ubiquitin and the tryptic peptides of maleated human ubiquitin

	Ubiquitin	MT-1	MT-2	MT-3	MT-4
Asp	6.9(7)	4.3(4)	2.0(2)	1.2(1)	0.1(0)
Thr	7.2(7)	4.6(5)	1.9(2)	0.2(0)	0.1(0)
Ser	2.9(3)	0.7(1)	1.7(2)	0.0(0)	0.0(0)
Glu	12.4(12)	7.6(8)	2.4(2)	2.2(2)	0.1(0)
Pro	3.4(3)	3.1(3)	0.2(0)	0.0(0)	0.0(0)
Gly	4.3(4)	2.2(2)	0.2(0)	2.3(2)	0.2(0)
Ala	2.2(2)	1.3(1)	0.1(0)	1.2(1)	0.1(0)
½ Cys	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
Val	4.2(4)	3.4(3)	1.0(1)	0.1(0)	0.0(0)
Met	0.9(1)	0.8(1)	0.1(0)	0.1(0)	0.0(0)
Ile	6.9(7)	4.8(5)	1.0(1)	1.1(1)	0.1(0)
Leu	9.1(9)	2.4(2)	4.0(4)	2.3(2)	1.0(1)
Tyr	0.9(1)	0.2(0)	0.9(1)	0.1(0)	0.0(0)
Phe	1.9(2)	1.0(1)	0.1(0)	1.0(1)	0.1(0)
Trp	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
Lys	7.2(7)	5.1(5)	1.1(1)	0.1(1)	0.1(0)
His	1.0(1)	0.2(0)	0.9(1)	0.0(0)	0.0(0)
Arg	4.2(4)	1.3(1)	1.0(1)	1.1(1)	0.9(1)
N-terminus	Met	—	Thr	Leu	Leu
Total	74	42	18	12	2

The composition of each peptide is given as the molar ratios of the amino acids; figures in parentheses are the assumed numbers of residues. These findings are identical to those for bovine ubiquitin[4].

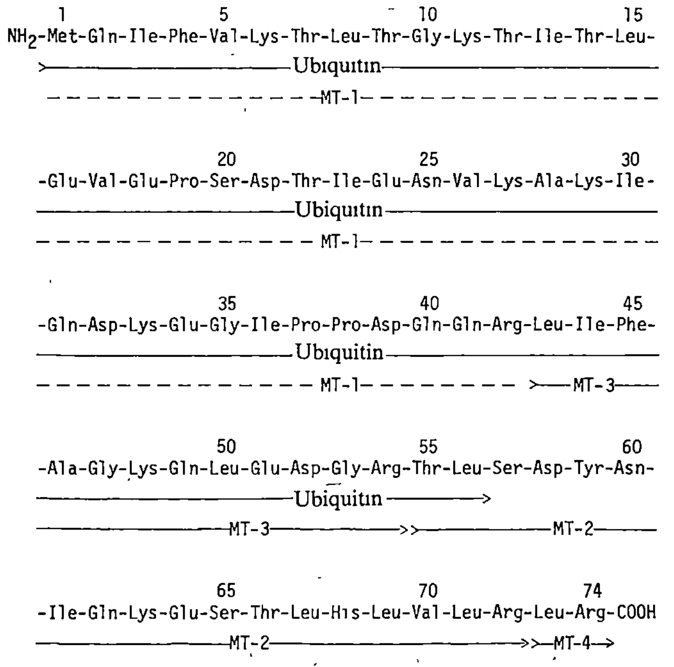
1 5 10 15
NH2-Met-Gln-Ile-Phe-Val-Lys-Thr-Leu-Thr-Gly-Lys-Thr-Ile-Thr-Leu-
Ubiquitin
MT-1
20 25 30
-Glu-Val-Glu-Pro-Ser-Asp-Thr-Ile-Glu-Asn-Val-Lys-Ala-Lys-Ile-
Ubiquitin
MT-1
35 40 45
-Gln-Asp-Lys-Glu-Gly-Ile-Pro-Pro-Asp-Gln-Gln-Arg-Leu-Ile-Phe-
Ubiquitin
MT-1
MT-3
50 55 60
-Ala-Gly-Lys-Gln-Leu-Glu-Asp-Gly-Arg-Thr-Leu-Ser-Asp-Tyr-Asn-
Ubiquitin
MT-3
MT-2
65 70 74
-Ile-Gln-Lys-Glu-Ser-Thr-Leu-His-Leu-Val-Leu-Arg-Leu-Arg-COOH
MT-2
MT-4

Fig. 1 Complete amino acid sequence of human ubiquitin. Arrows to the right indicate amino acids identified directly from Edman degradations with each arrow bearing a notation indicating the peptide studied (see text). The extent of peptide MT-1 is indicated by a broken line.

This work was supported by funds from the US Public Health Service and the Irvington House Institute. Ronald King, Miriam Miller and Philip Dee provided assistance.

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Received March 5, 1975

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Hybrid troponin reconstituted from vertebrate and arthropod subunits

TROPONIN, a regulatory protein modifying muscular contraction is found in all the chordate striated muscles as well as in many of the invertebrate muscles which have been studied[1–3]. The complex of troponin and tropomyosin is bound to the actin containing thin filaments, and both components are required for thin filament-linked regulation[1–3,10]. In the absence of calcium the troponin–tropomyosin complex blocks the myosin combining sites, inhibiting ATPase activity and therefore contraction. When present in micromolar amounts, calcium binds to troponin and releases the inhibition Vertebrate troponin consists of three subunits: TN-I, TN-C, and TN-T (ref. 4). TN-I by itself (molecular weight 24,000) inhibits the actin–myosin interaction irrespective of calcium ion concentration. TN-C (molecular weight 18,000) binds calcium ions and this releases the TN-I imposed inhibition. TN-T (molecular weight 37,000) attaches the subunit complex to tropomyosin. The troponin found on arthropod thin filaments is also composed of subunits[3,5,6]. Components with chain weights of 29,000 and 18,000 have features analogous to those of vertebrate TN-I and TN-C respectively[6]. A third component is also found in arthropod troponin preparations (chain weight 55,000–59,000), but its function is not yet fully understood[5,6]. For convenience, this chain will be referred to here as TN-T. In spite of the overall similarity of vertebrate and arthropod troponin, invertebrate troponin differs from vertebrate troponin in calcium binding properties, and binds one-half to one-quarter of the calcium in conditions where the thin filament is maximally switched-on[3,6]. Moreover, the thin filament-based regulation in invertebrates is usually coupled with a myosin-linked regulatory system which is apparently not present in chordate striated muscle[3,10].

It is thought that there was a divergence early in the evolution of the animal phyla leading separately to the chordates and to the segmented invertebrates. Since arthropods and vertebrates have separate lineages, the troponin in these phyla may represent examples of convergent evolution, or may have evolved from a common ancestor. One approach for distinguishing between these alternatives, is to determine whether or not interphyletic hybrids can be made and are functional. In this study I show that a functional troponin hybrid can be made from rabbit TN-I and TN-T and *Limulus* TN-C.

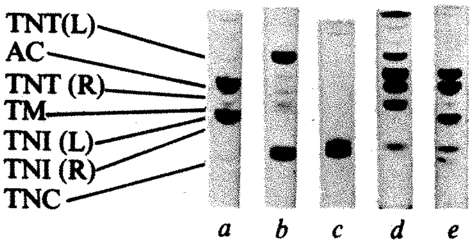

Fig. 1 10% SDS polyacrylamide gels[9] using Coomassie Brilliant Blue stain. *a*, Rabbit TN-I-T, 10 µg. *b*, *Limulus* TN-C-T, 11 µg. *c*, *Limulus* TN-C, 15 µg. *d*, *Limulus* thin filaments, 30 µg. *e*, Reconstituted thin filament complex of rabbit actin, chicken tropomyosin, rabbit TN-I-T, and *Limulus* TN-C (proteins mixed in a 1.0:0.5:0.5:1.3 weight ratio in 40 mM NaCl, 1 mM MgCl and sedimented at 160,000*g* for 2 h, and the pellet used for gel electrophoresis). This gel shows approximately 10 µg actin. (R) and (L) denote rabbit and *Limulus*, and AC and TM represent actin and tropomoyosin. All proteins can be resolved into separate bands except TM and TN-T(R) on gel *e*.

Rabbit actin, myosin, and chicken tropomyosin were prepared as previously described[8,7]. Rabbit and *Limulus* troponins were prepared according to Regenstein and Szent-Györgyi[6]. A fraction containing rabbit TN-I and TN-T (TN-I-T) (Fig. 1*a*) was prepared essentially by the method of Greaser and Gergely[4]. TN-I and TN-T were eluted together with the void volume from DEAE–Sephadex A-50 equilibrated with 100 mM NaCl, 2 mM EDTA, 6 M urea, 0.03% β-mercaptoethanol, 50 mM Tris *p*H 8.0. A preparation containing primarily *Limulus* TN-C and TN-T (TN-C-T) (Fig. 1*b*) could be prepared either by the method of Regenstein and Szent-Györgyi[6] or by a second method in which an equal weight of DEAE–Sephadex A-50 is added to the *Limulus* troponin and the mixture dialysed against the buffer described above. The Sephadex is sedimented and the TN-I remains in the supernatant. The TN-C-T can be removed from the Sephadex by sedimention in the same solution containing 0.6 M NaCl. Conventional methods used to prepare vertebrate TN-C, when applied to arthropods, yield a TN-C contaminated with variable amounts of TN-T. To prepare a purer TN-C (Fig. 1*c*) an equal weight of SP-Sephadex C-50 is added to the troponin and the mixture dialysed against 90 mM NaCl, 6 M urea, 0.03% β-mercaptoethanol, 10 mM sodium acetate *p*H 5.0. The Sephadex is sedimented and the TN-C is in the supernatant. This method was also used to prepare rabbit TN-C except that the *p*H of the buffer was adjusted to 6.0. The fractionated *Limulus* subunits and the rabbit TN-C were then dialysed against 40 mM NaCl, 1 mM $MgCl_2$, 5 mM phosphate buffer *p*H 7.0, and small amounts of precipitate were removed by centrifugation and discarded. The rabbit TN-I-T precipitates in these conditions and was redissolved in 0.6 M NaCl.

Table 1 ATPase activities of actomyosin preparations

Troponin subunits added	Mg-ATPase EGTA	Mg-ATPase Calcium	Calcium sensitivity $100-\frac{(\text{ATPase EGTA})100}{\text{ATPase Ca}}$
	(µmol ATP split per min per mg myosin)		
a, None	0.5	0.5	0
b, Rabbit TN-I-T	0.04	0.04	0
c, Rabbit TN-C*	0.47	0.47	0
d, Rabbit TN-I-T Rabbit TN-C*	0.14	0.57	75
e, *Limulus* TN-C-T†	0.33	0.30	0
f, *Limulus* TN-C‡	0.39	0.35	0
g, Rabbit TN-I-T *Limulus* TN-C-T†	0.12	0.37	68
h, Rabbit TN-I-T *Limulus* TN-C‡	0.19	0.39	52

In each ATPase assay 0.26 mg rabbit F-actin was mixed with 0.14 mg chicken tropomyosin in 40 mM NaCl, 1 mM $MgCl_2$, 5 mM phosphate buffer *p*H 7.0, brought to 0.4–0.6 M NaCl, and then mixed with 0.14 mg TN-I-T, or TN-C, TN-C-T or combinations of these at 0.4–0.6 M NaCl. Finally, 0.8 mg rabbit myosin was added. The mixture was then diluted in 10 ml 0.7 mM ATP, 1 mM $MgCl_2$, *p*H 7.5, 25 °C having a final NaCl concentration of 40 mM, and the ATPase assayed using the *p*H stat method as described previously[7,8]. The calcium dependence was determined by comparing the ATPase rates in 0.1 mM EGTA and in 0.1 mM $CaCl_2$.

*0.08 mg

†0.4 mg

‡0.3–0.6 mg (variation in range maximally effective in different preparations).

It is well established that the addition of rabbit TN-I-T to tropomyosin-containing actomyosin results in a calcium independent inhibition of ATPase activity (Table 1*b*) and calcium sensitivity is restored when rabbit TN-C is also added (Table 1*d*) (compare ref. 4). In these studies it was found that a functional troponin is reconstituted by combining these fractions in 0.4–0.6 M NaCl and it was not necessary to mix the proteins in urea solution. The TN-I-T can be repeatedly frozen and thawed without losing inhibitory activity or its ability to complex functionally with TN-C.

Functional troponin hybrids can be made by mixing rabbit TN-I-T with *Limulus* TN-C-T or with *Limulus* TN-C, and the hybrids confer a calcium dependence on tropomyosin-containing rabbit actomyosin (Table 1*g,h*). Actin and tropomyosin combine with the latter hybrid troponin to form a complex which is free of *Limulus* TN-T and TN-I (Fig. 1*e*).

The amount of *Limulus* TN-C necessary for forming functional hybrids with rabbit TN-I-T is greater than the comparable rabbit TN-C requirement (Table 1). At reduced *Limulus* TN-C levels only a fraction of the TN-I-T imposed inhibition is relieved, but this activity is calcium sensitive. These results may indicate that the *Limulus* TN-C has a lower affinity for rabbit TN-I-T than rabbit TN-C, or that some of the *Limulus* TN-C may bind to actin–tropomyosin nonspecifically. It is also possible that the *Limulus* TN-C preparation may be partly denatured; however, it should be noted that dialysis of whole troponin against 6 M urea at *p*H 5.0 does not diminish its activity, nor is the activity of *Limulus* TN-C altered by repeating its purification twice.

Since *Limulus* TN-C can substitute for rabbit TN-C and participate in forming a calcium sensitive rabbit actomyosin ATPase, it seems likely that the subunits of vertebrate and arthropod troponin function by the same mechanism and have therefore probably evolved from a common ancestor.

This work was supported by grants from the National Science Foundation and the US Public Health Service.

Note added in proof: A complementary experiment was performed in which chicken TN-C-T and lobster TN-I were mixed; a functional hybrid was formed which conferred calcium sensitivity on vertebrate actomyosin.

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Received March 4; accepted April 2, 1975.

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Self-assembly of lens crystallins *in vitro*

THE lens of vertebrate eye is very useful for the study of a number of fundamental biochemical processes at the molecular level. In this avascular tissue a continuous process of cellular differentiation takes place starting in the outer epithelial monolayer and ending in the lens centre, which consists of closely packed fibre cells. Strictly speaking, no cell death occurs in the lens, although differentiation from epithelium to fibre is accompanied by a gradual loss of cell organelles such as mitochondria, nuclei and ribosomes. Also, a very slow but defined breakdown of proteins starting from the C terminus[1,2], and deamidation of crystallins during ageing have been reported[3].

We have shown previously that after treatment with 7 M urea the major structural lens protein α crystallin (molecular weight about 800,000) could be dissociated into subunits of molecular weight 20,000. After removal of urea the correct reassociation of subunits was achieved[4,5]. Although the isolated β crystallin fraction could also be deaggregated, reassociation of this class of lens proteins failed, only insoluble aggregates being recovered. Eventually the complete mixture of all water-soluble lens proteins was also treated with urea and about 20 different monomeric polypeptides (20,000–30,000) were obtained. On dissociating the total mixture of 'native' crystallins, however, selective reaggregation into α, β and γ crystallin could not be achieved. Since the observation that even enzymic activity (for example, ribonuclease[6]) could be restored after complete unfolding with urea, renaturation could be achieved by removal of the denaturing agent under well defined conditions[6]. Wetlaufer and Ristow[7] stressed that the conformation of a protein depends on the kinetics as well as on the energetics of the folding process. On the other hand, the correct reassociation of groups of differing well defined protein assemblies in a complex mixture of highly hydrophobic components has not, to our knowledge, been reported previously. In a cell-free system derived from calf eye lens[7], it seems that α, β and γ crystallins were synthesised and sorted out into proper assemblies. As, after translation, the individual proteins are presumably released from the ribosomes as monomeric polypeptides, it should be possible to detect conditions which promote the correct assembly of the subunits. The present experiments show why we failed in 1962 and how 100% renaturation starting from a highly complex mixture of monomeric subunits can be achieved.

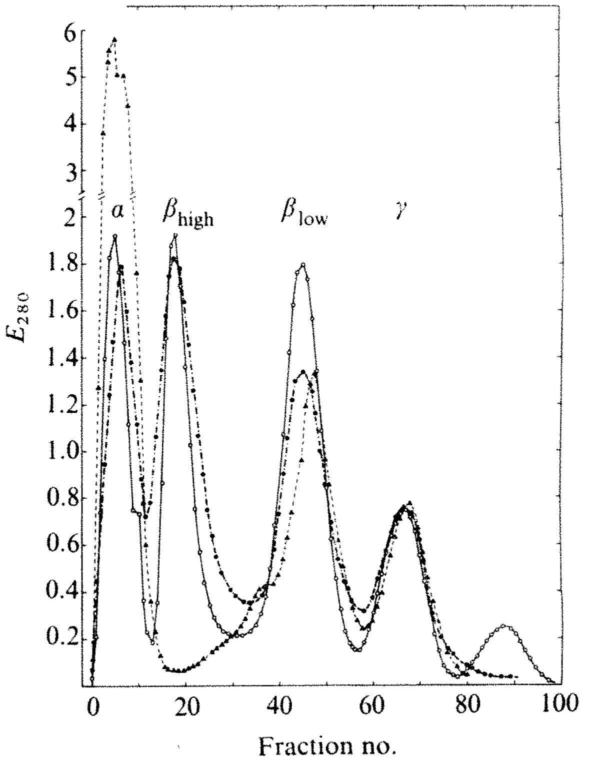

Fig. 1 Gel filtration patterns of native and renatured lens crystallins on Sephadex G-200. ○, Native crystallins; ▲, Crystallins reassociated at a protein concentration of 200 mg ml^{-1}; *Crystallins reassociated at a protein concentration of 2 mg ml^{-1}. Column dimensions 100 × 4 cm. Equilibration and elution buffer 0.05 M Tris-HCl containing 0.051 M NaCl and 0.001 M EDTA (ref. 12) at *p*H 7.6. Elution was performed at room temperature for 40 h. Flow rate 18 ml h^{-1}. Preparation of total water-soluble lens extract has been described previously[13].

The water-soluble lens proteins can be separated by gel filtration on a Sephadex G-200 column. Four fractions are obtained, the first peak eluting with the void volume being α crystallin, followed by β_{high}, β_{low} and γ crystallin (average molecular weights 800,000 150,000, 50,000 and 20,000, respectively; Fig. 1). When the mixture of water-soluble lens proteins (200 mg ml^{-1}, optimal for separation by gel filtration in our experimental conditions) is dissociated with 7 M urea, dialysed against large volumes of deionised water, lyophilised, redissolved in 0.05 M Tris-HCl buffer (*p*H 7.6) containing 0.05 M NaCl and 0.007 M EDTA, and reapplied to a Sephadex G-200 column, another separation pattern, as obtained with native lens protein, is observed (Fig. 1). The β_{high} fraction has disappeared almost completely and β_{low} fraction has been significantly decreased. The peak fractions from the separations depicted in Fig. 1 were isolated and subjected to electrophoresis in polyacrylamide gels containing 7 M urea to obtain the subunit patterns shown in Fig. 2.

Reaggregation has clearly resulted from complexing of α and β components (Fig. 2*b* and 2*d*). γ Crystallin is not involved in complex formation (Fig. 2*h*). These results were substantiated by electron microscopy and immunochemical studies[9]. Of the various conditions for correct reassembly studied (such as *p*H, temperature, time dependence or dilution after removal of urea) only the protein concentration at the moment of urea removal determined proper or incorrect assembly. Thus, if a mixture of the naturally occurring crystallin polypeptides at any concentration between 200 and 20 mg ml^{-1} was treated with 7 M urea the only prerequisite for correct recombination was dilution of the protein solution with 7 M urea to a final concentration of 2 mg ml^{-1} before dialysis (Fig. 1). The gel

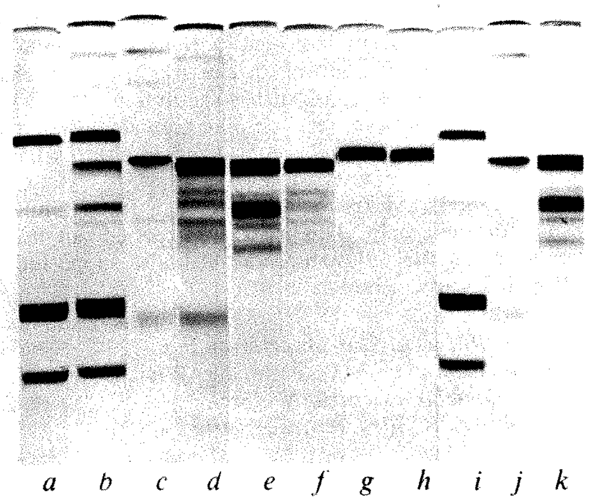

Fig. 2 Electrophoretic analysis of the separated water-soluble lens proteins on 7.5% polyacrylamide gels containing 7 M urea. *a*, Native α crystallin; *b*, $\alpha\beta$ hybrid; *c*, native β_{high}, *d*, $\beta_{\text{high}}\beta_{\text{low}}$ hybrid; *e*, native β_{low}; *f*, β_{low} reassociated; *g*, native γ; *h*, γ reassociated (note that only the γ fraction is not involved in complex formation); *i*, correctly reassembled α crystallin fraction; *j*, correctly reassembled β_{high} crystallin fraction; *k*, correctly reassembled β_{low} crystallin fraction. *b*, *d*, *f*, and *h* show the electropherograms of α, β_{high}, β_{low} and γ crystallin reassociated at 200 mg ml^{-1} protein and *i*, *j* and *k* at 2 mg ml^{-1}. γ Crystallin, which in all cases is unaltered, is not shown. Electrophoresis was performed as described elsewhere[14].

filtration pattern shows that all crystallin components have formed. Gel electrophoresis proves that these renatured crystallins contain the correct polypeptides and that no hybrid formation took place. The actual concentration of the individual polypeptide chains therefore seems to provide a rather trivial but efficient regulatory mechanism for correct *in vitro* assembly of high polymeric proteins. Moreover, the results can also be interpreted by assuming that refolding of the monomeric polypeptides at low concentration is a first order reaction whereas the rate of interchain interaction is of a higher order. In this case proper interchain interaction of all crystallin polypeptides precedes the recombination process. Such a mechanism has recently been reported for renaturation of the hormones, bovine thyrotropin[10] and ovine interstitial cell stimulating hormone[11].

Note that the selective recombination cannot be achieved after dissociation of the lens proteins into subunits by either acid or alkaline treatment. On neutralisation even at extremely high dilution only nonspecific reaggregation of α and β crystallin chains could be observed.

In vivo, another mechanism may be operative. If one assumes that proper folding of the peptide chains takes place while still on the ribosomes, correct assembly of the individual chains into the native polymer would occur. If, however, the folding of the individual chains occurs after release from the ribosomes the proper assembly may be regulated by the actual (low) concentration.

Our findings, although providing the tool for correct *in vitro* assembly, do not allow definite conclusions for the *in vivo* situation to be drawn.

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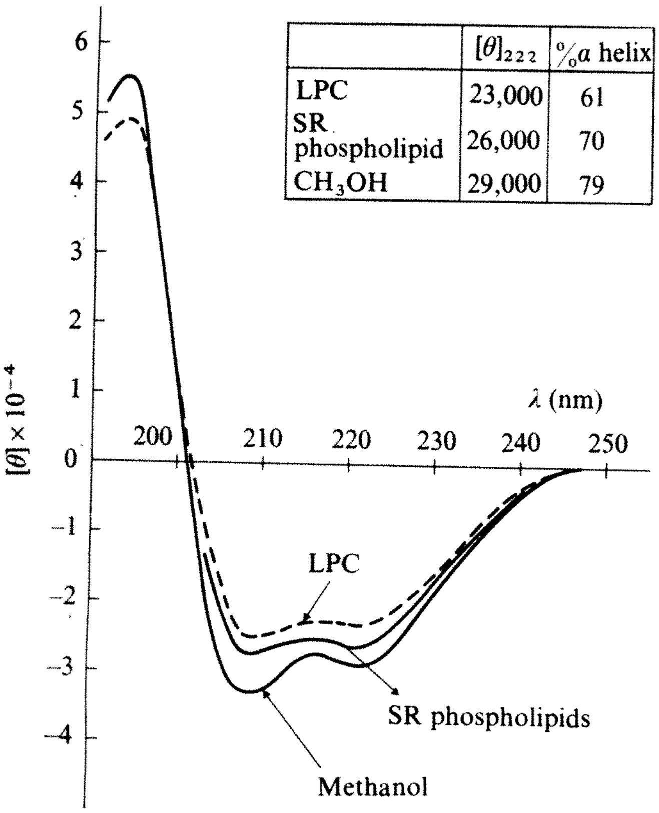
A highly α-helical structure protein in sarcoplasmic reticulum membranes

AMONG the various protein fractions isolated from sarcoplasmic reticulum (SR) membranes, MacLennan *et al.*[1] demonstrated the existence of a proteolipid of molecular weight about 12,000, that is, a protein which is soluble in 2 : 1 chloroform–methanol mixture[2,3]. Recent spin-labelling (P. L. and M. D. Barratt, unpublished) and black-film experiments (P. L. and D. E. Graham, unpublished) have provided evidence that the proteolipid resides in the hydrocarbon interior of the phospholipid bilayer and reduces the alkyl-chain mobility. The apparent lack of any specific action in the Ca^{2+}-transport process of SR suggests that the proteolipid merely provides structural support, possibly by stabilising the vectorial character of the transport system within the dynamic phospholipid matrix. Considering the widespread presence of proteolipids in biological membranes[3], structural information about this type of intrinsic protein is of particular interest. With intrinsic membrane proteins the choice of methods for obtaining such direct information is restricted by the fact that in the native state the protein is associated with lipid molecules in aggregates of comparatively large dimensions. In this situation data on the secondary structure from measurement of the circular dichroism may be used to infer a model for the arrangement of the protein within the membrane.

Methods of preparing proteolipid from rabbit skeletal muscle, SR phospholipids and recombined systems were essentially as described previously[1,2]. Circular dichroism spectra in the far ultraviolet range were obtained using proteolipid in methanol, and in aqueous dispersions of SR phospholipids and lysophosphatidylcholine (LPC). The micellar and vesicular character of the LPC and sonicated SR-phospholipid dispersions was confirmed by negative stain electron microscopy. The average Stoke's radius of the proteolipid-containing SR-phospholipid vesicles (by Sepharose 4-B column chromatography[4]) was 76 Å. Because of this small particle size no corrections[5,6] for differential light scattering and absorption flattening were made, because at wavelengths higher than 200 nm such effects would be rather small and hardly affect conclusions drawn.

Comparison of the spectra (Fig. 1) with those of model polypeptides of known conformation[7] reveals the typical features of a predominantly right-handed α-helical conformation in all three systems. Absorption flattening may be responsible for the relatively low positive maximum near 195 nm, although there seems to be no redshift caused by the particulate nature in the aqueous dispersions. Thus, an estimation of the α-helical proportion can be made from the ellipticities at 222 nm (ref. 8). The resulting values (Fig. 1, insert) are distinctly higher than those of most globular, water-soluble proteins[9] in all three environments. The fact that the α-helical character is maintained in



	$[\theta]_{222}$	% α helix
LPC	23,000	61
SR phospholipid	26,000	70
CH_3OH	29,000	79

Fig. 1 Far ultraviolet circular dichroism spectra of SR proteolipid in methanol and in aqueous dispersions of membrane phospholipids and LPC (from egg yolk lecithin, Lipid Products, South Nutfield, UK), measured on a Cary 60 spectropolarimeter equipped with a Cary 6001 CD-accessory. Blanks measured with solutions of all components except the proteolipid were substracted. The molar ellipticities [θ] in deg cm^2 dmol^{-1} were calculated from $[\theta]$ = mean residue weight × $(\theta/10lc)$, where θ is the observed ellipticity, l is the optical path length in cm, c is the protein concentration in g cm^{-3} determined by the Lowry[11] method using bovine serum albumin as a standard, and the mean residue weight was taken as 119.3 (calculated from the amino acid composition[1]). Weight ratios of protein–SR phospholipids, and protein–LPC were 1:50 and 1:20, respectively. Dispersions of SR phospholipid and proteolipid were sonicated under N_2 and ice-cooled for 20 min in volumes of 1 ml using a microsoniprobe (MSE, England). The α-helical fraction was calculated from $[\theta]_{222}$ according to Glaser and Singer[8].

methanol solution indicates that the conditions of proteolipid preparation do not interfere severely with the native conformation. Considering that the α-helical regions are essentially of cylindrical or rod-like shape, the relatively low α-helix fraction in the LPC-system can be rationalised on the grounds of the spherical micellar structure of the aggregates, which does not allow the formation of extended α-helical stretches within its restricted low-polarity core. No such hindrance prevails in the bilayer structure of the SR-phospholipid vesicles and, correspondingly, the α-helical proportion is higher there.

These results support the hypothesis that in the SR membrane the proteolipid is arranged predominantly in extended cylinders of α helices in the plane of the bilayer. The formation of a two-dimensional network of aggregated proteolipid molecules into which the phospholipid alkyl-chains are interdigitated would also explain the observed physical effects (P. L. and M. D. Barratt; and P. L. and D. E. Graham, unpublished) of a protein of relatively small molecular weight on a high number of phospholipid molecules.

It is likely that similar structures are present in other biological membranes containing proteolipid. The finding of Racker and Kandrach[10] showing that a hydrophobic protein fraction from inner mitochondrial membranes, which by itself has no apparent biological activity, is essential to the functional reconstitution of an electron transport chain underlines the importance of such structural proteins. This type of intrinsic protein, of two-dimensional rather than corpuscular structure, may be considered in the discussion of current membrane models.

I thank Drs E. Morris and O. Korver from Unilever Research for their help. This work was carried out in the course of an EMBO fellowship, and was also supported by The Royal Society, London.

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Received February 10; accepted March 27, 1975.

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Errata

In the article "Chromosome imprinting and the mammalian X chromosome" by H. Sharat Chandra and S. W. Brown (*Nature*, **253**, 165; 1975), page 168, paragraph 2, line 1, for ref. 33 read ref. 32.

In the article "Energy spectrum of diffuse component of cosmic soft γ rays" by Y. Fukada, S. Hayakawa, I. Kasahara, F. Makino, Y. Tanaka and B. V. Sreekantan (*Nature*, **254**, 398; 1975) the ceiling heights given for the balloon in the fourth paragraph should read 430 and 680 Pa and not as printed.

Corrigenda

In the article "*Paracoccus denitrificans* and the evolutionary origin of the mitochondrion" by P. John and F. R. Whatley (*Nature*, **254**, 495; 1975) there is an error in the legend to Fig. 2. For Tris-acetate read Tris-phosphate.

In the article "Missing link between Milankovitch and climate" by G. J. Kukla (*Nature*, **253**, 600; 1975) the *Q* values in Table 1 are incorrect. The correct values are shown in the table reprinted below. These errors in no way affected the text, figures and conclusions that followed in the original article.

Table 1 Monthly means* of *Q*, *T* and *S*

	Globe			Northern Hemisphere			Southern Hemisphere		
	Q langley d^{-1}	*T* °C	*S* 10^6 km^2	*Q* langley d^{-1}	*T* °C	*S* 10^6 km^2	*Q* langley d^{-1}	*T* °C	*S* 10^6 km^2
January	566	12.2	76	380	8.0	58.4	751	16.5	18
April	547	14.1	59	644	14.2	41.2	451	14.1	18
July	536	16.1	42	723	21.6	14.3	349	10.6	28
October	548	14.3	57	480	16.2	22.8	616	12.4	34
Mean	550	14.2	60	556	15.0	34.8	543	13.4	25

**Q*, After Berland[10]; *T*, long term means after Shutz and Gates[11]; satellite observed mean *S* areas covered for at least five consecutive days in the Northern Hemisphere during 1967–73 interval and in the Southern Hemisphere in 1968 (ref. 14)

matters arising

No evidence for heritability of social attitudes

Eaves and Eysenck[1] have concluded that six types of personality orientations, including both radicalism and neuroticism, have substantial genetic components. These conclusions, based on simple models, are presented with some qualifications but not without conviction and certainty. The reader is informed that: "a model invoking only environmental factors fails to provide an adequate description of the variation and covariation of the six traits"; that, although a larger study would be desirable, "we can, however, be reasonably confident that some kind of genetic mechanism has to be involved to account for the observations so far"; and that the correct interpretation of a table presenting exact heritability estimates for each trait, is that "about half the variation between individuals for the six traits has a genetic basis".

None of these conclusions is justified by the data. Eaves and Eysenck's two-parameter environmental model tests only whether dizygotic twins are as similar in their personality scores as monozygotic twins. The high χ^2 for this model indicates that the monozygotic twins are more similar than dizygotic twins. Eaves and Eysenck simply assume that this extra similarity is genetic. In a paper[2] on the heritability of IQ performance we showed that comparisons between monozygotic and dizygotic twins can only set upper limits to the heritability of traits because of the greater similarity of treatment of monozygotic twins. We estimated the magnitude of this treatment effect and showed it to be comparable with the presumed genetic effect.

Eaves and Eysenck make the same error in the present study. They have assumed that monozygotic twins, who look alike and are regularly confused for each other, receive the same treatment as dizygotic twins who are rarely confused[3] and they assume that the rejection of a primitive two-parameter environmental model means that no environmental model can be fitted to the data. Such procedures are inadequate. Comparisons between dizygotic twins of the same sex and dizygotic twins of the opposite sexes, for example, should be reported to see if the differences are comparable with monozygotic–dizygotic differences. If so, this would indicate that environmental effects could account for greater monozygotic similarity and indicate an absence of genetic effects. Even better would be to compare dizygotic twins who look alike[3] with monozygotic twins, to control for physical similarity.

The models used in this study and in IQ studies[4] have come to substitute for sensible experimental controls and for adequate tests of competing hypotheses. The conclusions are unwarranted and misleading. They reflect only the assumptions of the authors and assume the very results they are trying to prove.

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Specificity in initiation of mRNA translation

Lodish[1] proposes a model for the regulation of mRNA translation in which changes in specificity may actually be provoked by a nonspecific change in a kinetic parameter of protein synthesis. I had developed a similar idea earlier[2,3] in the discussion of ribosomal discrimination at the elongation level[4] whereas Lodish discusses the initiation step. The mathematics of Lodish's paper seem to be inaccurate, in addition to the acknowledged simplifications.

Equation (1) applies to infinite chains only. Equation (3) cannot be correct; this can be checked by solving the steady-state equations in a simple case (for example, a messenger three codons in length where $L=2$). Combining these two leads to equation (5), which predicts negative rates of protein synthesis when initiatable ribosomes are in large excess ($K_e/K_1R^* < 1$). Lodish does not claim that his treatment is valid for such extreme cases. The presence of the approximations casts doubt on the claim that the treatment takes into account the effect of hindrance to translocation of a ribosome as a result of the presence of an adjacent ribosome. When this effect is negligible, the correct rate equation can be established very simply. Consider an initial situation in which a ribosome has just moved from the Lth to the $(L+1)$th codon. The mRNA becomes initiatable. The average time which elapses before there is a collision with a ribosome is, by definition of the kinetic parameters of Lodish's model, $1/R^*K_1$. Completion of initiation then takes a certain time ε, and the L successive translocations take, by definition, a time L/K_e. The mRNA is now initiatable again and a new cycle begins. Thus, the average time (that is the reciprocal of the turn-over number) it takes for a mRNA molecule to produce a protein is:

$$t = 1/R^*K_1 + \varepsilon + L/K_e \qquad (1)$$

When ε is negligible, equation (1) can be written as:

$$QL/mK_e = L/tK_e = K_1/(K_1 + K_e/R^*L) \qquad (2)$$

This kinetic treatment of specificity in initiation of translation is isomorphous with my treatment of elongation (3) in which I had:

$$\rho=\theta/(\theta+\tau) \qquad (3)$$

where θ (like K_1) characterises the strength of the association and τ (analogous to the R^* term) is the parameter that changes nonspecifically. A more sophisticated model has been considered recently[5].

Substituting $(L-1)$ with L in the denominator of the right-hand expression of Lodish's equation (5) one gets my equation (2).

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Dr Lodish replies—Ninio's[1] equation (1) is incorrect on several grounds. First, it yields not the average time for an mRNA to produce a complete protein, but rather the average time it takes an mRNA to produce a polypeptide L amino acids long (L being the number of codons covered by a single ribosome). Secondly, it neglects, by his own admission, the case in which progress of one ribosome is hindered by the presence of another distal to it along the mRNA. As is clear from Fig. 1 of my paper[2], this parameter is considerable for usual densities of ribosomes along an mRNA such as occur in (uninhibited) reticulocytes. The fact that Ninio can transform his equation into one, his equation (3), which resembles my equation (5), does not prove that his equation is correct.

I did point out in my paper the several simplifying assumptions that were made in my derivations. In particular, I pointed out that the equations were valid only for cases in which the overall rate of chain initiation is the same or less than that which obtains in a normal cell. Thus, Ninio's comments about cases in which initiatable ribosomes are in large excess ($K_1R^*/K_e > 1$) are irrelevant.

All my assumptions, although valid for reticulocytes, might not be for other types of cells. To remove these assumptions necessitates a much more complex statistical analysis of ribosome movement. These are in progress in my laboratory. But I am afraid Ninio's equations are incorrect and do not really help matters.

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Normal redshifts in Markaryan galaxies

Teerikorpi claims to have found evidence for anomalous redshifts in Markaryan galaxies. His method was to take a sample consisting of the 394 measured redshifts of galaxies in Markaryan's first five lists. He then divided the redshifts into bins of $\Delta z = 0.001$, and, according to him, a peak in the resulting distribution at $z = 0.015$–0.016 is too large to be a chance effect.

Teerikorpi estimates the significance of this peak by calculating the probability that 30 or more redshifts would occur in an interval of $\Delta z = 0.002$, assuming that the distribution of redshifts between $z = 0.003$ and $z = 0.030$ is rectangular, and assuming that Poisson statistics hold. Teerikorpi estimates this probability as 0.05. This is incorrect. There are 269 galaxies in 27 bins of $\Delta z = 0.001$, or approximately 20 galaxies for each bin of $\Delta z = 0.002$. The probability, with these assumptions concerning the distribution, is 0.02 that 30 galaxies will be found in a given interval; however, there are 26 such bins, making the true probability 0.44 that this is just a chance distribution. Things are no better if one considers intervals of $\Delta z = 0.001$. The probability of 17 or more in a given bin is 0.03; for 27 bins it is 0.52. Including the peak at $z = 0.006$, which Teerikorpi does not mention as significant, the probability that the observed distribution results by chance is 0.28.

Teerikorpi argues that 0.015 is an integral fraction of the peaks at $z = 0.03$ and $z = 0.06$ found by Burbidge[2] and by Burbidge and O'Dell[3,4]. No peak is found at 0.030 in Teerikorpi's sample, although it extends past that point, and no peak at 0.015 was found in the sample of galaxy redshifts used by Burbidge and O'Dell[3] even though more than 40% of their sample were Markaryan galaxies.

The assumptions about the gross distribution of redshifts are poor. It is not rectangular, but is a curve peaked near $z = 0.020$. This is exactly as expected with the observed counting statistics as a function of limiting magnitude if one assumes that the distribution of Markaryan galaxies in space and brightness is similar to normal galaxies. Also, as pointed out by Huchra and Sargent[5], Markaryan's survey does not yet cover a large enough area of the sky to remove adequately the small scale effects of clustering. Both of these effects will tend to decrease the significance of any clumps or peaks in the redshift distribution. Finally, because of the small number of samples (27 bins), and the interdependence of the samples, (a peak in one bin will cause a dip in others, with a fixed number of galaxies), it is not clear that Poisson statistics should be used. If a distribution with a larger half width is used, then the significance of the peaks is decreased still further.

In the light of these arguments, I find nothing to support the claim for anomalous redshifts or non-cosmological components for Markaryan galaxies.

This work was supported in part by the NSF.

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[5] Huchra, J., and Sargent, W. L. W., *Astrophys. J.*, **186**, 433–443 (1973).

Dr Teerikorpi replies—My incorrect value resulted from an unfortunate elementary error in my calculations[1], and went unnoticed till now. I am grateful to Huchra for drawing my attention to it.

The low statistical significance of the peak does not, however, invalidate altogether my other arguments, which were the main reasons for suggesting anomalous redshifts. The peak as such was only marginally significant.

It is still interesting that this peak, which at the first sight is a natural candidate for a possible indicator of anomalous redshifts, is consistent with simple ideas concerning intrinsic redshifts: they tend to occur in compact objects, they should be more easily detected in the direction of near, dense galaxy populations, and they possibly prefer certain values connected with simple relationships. One cannot compare the results of Burbidge and O'Dell[2,3] with my analysis. I did not divide the objects according to their radio or emission-line properties, only according to the ultraviolet classification of Markaryan. My sample also contained at least four times as many Markaryan objects as theirs did. Their first analysis of emission-line objects did not include any Markaryan galaxies in the relevant interval: 0.01–0.02.

It is harder to detect relatively small anomalous redshifts than large ones (such as in QSOs, if real) because in addition to the smearing factors I mentioned (random motions, cosmological recession) there are in this case many normal Dopplerian redshifts as 'noise' in redshift distributions. So in order to find out those properties which indicate intrinsic redshifts in objects one must work with a lower statistical significance level and use additional consistency tests. Otherwise possible anomalous redshift 'signals' may well go unnoticed.

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[1] Teerikorpi, P., *Nature*, **252**, 110–111 (1974).
[2] Burbidge, G. R., and O'Dell, S. L., *Astrophys. J.*, **178**, 583–605 (1971).
[3] Burbidge, G. R., and O'Dell, S. L., *Astrophys. J. Lett.*, **186**, L59–L62 (1973).

reviews

Cholinesterase end to end

J. Massoulié

The Biology of Cholinesterases. Frontiers of Biology, vol. 36.) By Ann Silver. Pp. xiv+596. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) Dfl.145; $55.95.

THE bible on cholinesterase has for more than 10 years been the voluminous *Handbuch des Experimentellen Pharmakologie,* edited by G. B. Koelle in 1963. Dr Silver's book is very different in many ways, mainly because it is the work of a single author. Although enzymological aspects of cholinesterase fall within the scope of the book along with more physiological points, the different chapters are all clearly oriented towards the biology of cholinesterases. The title of the book therefore describes its contents quite aptly.

Following Lewis Carrol's advice to Alice in Wonderland ("Begin at the beginning, and go on till you come to the end: then stop") which she uses as an introductory quotation, Dr Silver, after a few paragraphs on the history of cholinesterase research describes the enzymological and biochemical properties of the enzyme. This chapter is written in a way that will be easily comprehensible to students or scientists who are not familiar with enzymology. The meaning of the catalytic parameters is explained, and chemical terms are defined in a glossary at the end.

After a clear description of neuronal morphology, the subcellular localisation of acetylcholinesterase is covered, as well as its transport along the axon, and the location of its biosynthesis. Cholinesterases in invertebrates are discussed and an abundance of data on histological distribution of cholinesterase in vertebrates is then presented in a series of detailed tables (120 pages).

Three chapters are devoted to a discussion of the biological role of cholinesterase. Nachmansohn's theory of a universal cholinergic conduction mechanism is discussed. Dr Silver then considers the question: what is the function of acetylcholinesterase in erythrocytes, in placenta, and in nervous tissue outside cholinergic synapses? She studies the factors affecting its activity: the level of acetylcholinesterase varies with the physiological condition of the animals; it is under endocrine influence, and can be altered by drugs, lesions or other experimental interference; evidence points to a possible control of acetylcholinesterase by acetylcholine at motor endplates.

The last two chapters are devoted to pseudocholinesterases, and anticholinesterases which, quite logically, have been placed at the end.

I have to mention particularly the chapter on methods, which gives a precise and very detailed description of biochemical assays and histochemical localisation procedures for cholinesterases. Variants of these methods are carefully compared, and this part of the book should be very useful.

Research on acetylcholinesterase has been progressing very actively in the last few years, and it was inevitable that some of the more recent acquisitions could not be incorporated: recent data, for example, on the molecular structure of electric eel and torpedo acetylcholinesterase, or on the relationship of a definite molecular form with neuromuscular endplates in the rat diaphragm could not be incorporated although they would have been quite relevant. Neither have the new methods of acetylcholinesterase purification based on specific ligand chromatography been described. But I think that the book has succeeded in its aim to provide for the physiologist an introduction to the biochemistry of cholinesterase and for the biochemist an introduction to the relevant physiological problems.

Dr Silver's book, although not perhaps as exhaustive as Koelle's treatise, will act as an extraordinary source of information: it includes more than 1,800 references. Much of the data are presented in tables allowing that the text remains free for discussions, and rendering it very readable. It must have been a difficult task to organise heterogeneous data in this form, but it provides an easy method of retrieving information, and allows useful comparisons. A subject index is provided, but an author index would also have been useful.

Although the book is not meant to be read from beginning to end at one time, it is written in a simple and pleasant way, and is a valuable acquisition for any scientist concerned with cholinesterase and cholinergic transmission.

Complexones

Membrane-Active Complexones. By Yu. A. Ovchinnikov, V. T. Ivanov and A. M. Shkrob. Pp. xii+464. (Elsevier Scientific: Amsterdam, Oxford and New York, 1974.) Dfl135; $51.90.

THIS is a timely book, to be welcomed by workers and referees of papers in a field of widely dispersed literature. About half of it describes the constitution of natural and synthetic compounds which form lipid-soluble complexes with alkali and alkaline earth metal cations and stresses the vital importance of the three-dimensional structures in conferring the characteristic properties. Short chapters are devoted to methods of studying complex formation, with tables of results, and to possible uses of the phenomenon in chemistry which show how well Pedersen in 1967 signposted these aspects.

It is a genuine book, not a collection of papers, and has cohesion and perspective; it is written with the same lucidity as the original papers by the authors and their coworkers. Where appropriate they start with a description of their own work and then review related topics; this approach is successful and provides painless and excellent coverage of the literature up to and including 1972 in 1,111 references arranged in alphabetical order with a further 212 references, with titles, included to cover a few earlier papers and most of those published in 1972 and 1973. There is a good subject index.

It is unfortunate that many diagrams are reproduced from the literature and not redrawn by the excellent hand at the Shemyakin Institute because points which were obscure in the original remain so and errors are perpetuated; for example, Figure 141 shows the incorrect, absolute configuration of monensin from a preliminary communication. The sources of these diagrams are not clearly acknowledged giving a touch of irony to the publisher's almost hysterical note for forbidding reproduction. **M. R. Truter**

Biogeography

Plant Geography. (The Field of Geography) By Martin C. Kellman. Pp. xv+135+12 plates. (Methuen: London, January 1975.) £3.20 boards; £1.50 paper.

Terrestrial Environments. (Biology and Environments.) By J. L. Cloudsley-Thompson. Pp. 253. (Croom Helm: London, March 1975.) £5.95 boards; £3.25 paper.

THESE two books represent very different approaches to the task of conveying to the student in a concise form some elementary introduction to the study of biogeography. When confronted with this task, an author may present an account of general principles, illustrated by carefully chosen examples, or he may prefer to document as much factual information as possible and then attempt to stimulate the student into the formulation of general concepts for himself. Both approaches have their dangers; the former may result in an unbalanced coverage, whereas the latter may be indigestible.

Kellman's book has an honest, if traditional, title. It describes and illustrates what the author regards as the more important principles of plant geography. He begins, very sensibly, with a consideration of those aspects of the environment which influence the geographical distribution of a species both at the continental and at the habitat and microhabitat level. The importance of scale in plant geographical study is thus emphasised very effectively. Plant mobility is then considered very briefly, but could have been more profitably fused with a later chapter on terrestrial vegetation history, which protrudes rather prominently from a very mixed section labelled 'Other themes in plant geography'.

Another chapter within the 'Other themes' section covers the ecosystem concept. It is very difficult for biogeography texts to avoid such a chapter in these days as systems analysis, along with multivariate statistical analyses, are currently regarded as a panacea by many biogeographers. I do feel, however, that the relevance of the concept to plant geographical studies could be conveyed more convincingly, perhaps in connection with human management of plant species and vegetation in general. A large, central section of the book deals with vegetation analysis from a practical point of view and is concerned chiefly with methods of the description of vegetation at the habitat level. Many books already exist which cover this subject in far greater detail.

Plant Geography is one of a series of university level geography texts which are designed to introduce modern developments in geographical studies and which market at a low price. Judging the book in this light and regarding it as lead towards more detailed and specialised literature, it can be considered a successful enterprise.

Cloudsley-Thompson's latest book illustrates the other approach to the transmission of biogeographical information. Its title, *Terrestrial Environments*, is trendy but misleading. It is a book about animal geography, so why not entitle it accordingly? The basic approach is encyclopaedic; the author deals with each of the major biomes in turn and provides an anecdotal, annotated list of the more interesting animals occurring within them, concentrating upon the vertebrates. Vegetation, particularly its structural aspects, is dealt with briefly and mainly from the point of view of climatic adaptation and its potential as a source of animal microhabitats.

As a botanist, I find much of the information fresh and interesting, but I wonder whether the author falls between two stools. The information-packed nature of the text, together with the fair sprinkling of unexplained technical jargon, make difficult reading. The information given is often lacking in depth and, in spite of the very extensive bibliography, is likely to leave the enquiring student somewhat dissatisfied. Given patience and a good library for supplementary reading, however, the book could be useful. The final chapters, on such biogeographical/ecological principles as successional developments of habitats and population regulation, do not deal at all adequately with these subjects. If the author's hope is that the student who has assimilated the text will be in a position to formulate his own concepts, I fear that I disagree with him. However educationally improper it may seem, I feel that the average undergraduate needs to be fed principle as well as fact, and that factual information needs careful selection and predigestion. The practical limitations of teaching time usually force such an attitude upon us. On the whole my sympathy lies with Kellman's approach.

Peter D. Moore

Broad telescopic view

The Astronomical Telescope. By Boris V. Barlow. Pp. viii+213. (Wykeham: London and Winchester, January 1975.) £3.25 cloth; £2.50 paper.

THE large modern telescope is as much a product of engineering as optical science, so it is fitting that this excellent new book on telescopes has been written by B. V. Barlow, a mechanical engineer specialising in design specification. It has been written with the science student at universities and polytechnics in mind but it is an ideal introduction for any astronomer interested in the design and capabilities of optical telescopes. After an introduction to the history of the telescope and basic concepts in optics, image formation, interference and aberrations, the chapters deal with eyepieces, seeing and instrumental losses; telescope engineering, mirror production, mountings, tube and cell deflections and axis bearings; drive, time-keeping and computer control; ancillary equipment, spectrographs, image slicers, interferometers, photometers and bolometers; site selection and observatory buildings. There is a review of large telescopes, including the 200-inch Palomar, the Isaac Newton, the 60-inch infrared flux collector at Tenerife the Anglo-Australian, The AURA and the 6-m USSR. Extraterrestrial telescopes are also considered and Stratoscopes I and II, ESRO's ultraviolet telescope, and the NASA large space telescope are discussed. The book ends with a chapter on new developments in the use of telescopes. It is well indexed with a useful glossary and reference section, and is profusely illustrated with photographs and line drawing.

The astronomy community has needed a book like this for a long time and Mr Barlow is to be congratulated on his excellent achievement.

David W. Hughes

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Petroleum geology

Introduction to Petroleum Geology. By G. D. Hobson and E. N. Tiratsoo. Pp. viii+300. (Scientific Press: Beaconsfield, March 1975.) £10.50; $28.50.

OVER the past generation the greater number of textbooks on petroleum geology has originated in the USA, and the production of a well compiled and authoritative work by British authors is particularly to be welcomed at the time when the subject has achieved such a degree of national importance in Britain. Both authors have had long experience in this field.—G. D. Hobson on the academic and research side, E. N. Tiratsoo as a consultant with close involvement in the Middle East and Caribbean—and their combined expertise has been well applied.

The book covers the whole range of petroleum geology within the realms of exploration and production techniques, from the origin, accumulation and migration of petroleum, through exploration methods, formation logging and evaluation, the development problems, reserves assessment and—briefly—world-wide resources. It demonstrates a degree of assimilation of literature in this sphere which very few professionals would be able to match, and although the book is rather modestly stated to be "written to meet the requirements of students" there must be very few active geologists who would not find new and unfamiliar facts in its pages.

Although for the most part the book is adequately and clearly illustrated, it is unfortunate that a few of the diagrams (possibly reproduced in monochrome from coloured originals) are only legible with difficulty, and this might be given attention when a new edition is required.

Inevitably, some of the subjects described are controversial, but in such cases the authors have taken care to express both points of view, usually without taking sides.

There are a few points of special criticism: the discussion of map scales (p.135) shows a surprising absence of reference to the now almost universal use of metric values, the recommendation of mapping on 3-inches to a mile for example, is a remarkable idiosyncracy. Some miscellaneous data are irrelevant or unnecessary—such as the different values of the gal at the poles and equator, (p.152) and the description of plane table equipment (p.137)—but, overall, the selection of information is appropriate. One may hope that in a later edition the section on seismic surveys might be expanded, but the book gives an adequate if rather short summary.

Hobson and Tiratsoo have produced a volume which should have a place on the shelf of all serious petroleum geologists and others concerned with the technical problems of exploration and development of hydrocarbon resources; it will serve both as a very effective conspectus and as a guide to the extensive literature. **P. E. Kent**

Scattered electrons

High Energy Electron Scattering. (American Chemical Society Monograph.) By R. A. Bonham and M. Fink. Pp. vi+311. (Van Nostrand Reinhold: New York and London, June 1974.) £12.25.

THE content of this book is not as wide ranging as its title implies: the authors state in Chapter One that their interest is solely with high energy electron scattering from gaseous targets. Nevertheless, it does provide a comprehensive and lucid account of that branch of the subject, with emphasis given to the measurement of charge and momentum distributions in atoms and molecules.

The opening chapters contain a standard treatment of the relevant scattering theory and a review of theoretical methods of calculating atomic and molecular wave functions. Details are given of how elastic and total scattered electron intensity measurements may be used to obtain information on the radial distribution function and electron-pair correlation function of atoms; the experimental observations of electron correlation effects in atoms are discussed at length. The book continues with an account of the methods available for measuring the one and two-electron density function of molecules, and the results obtained from a number of molecules serve as useful illustrations. The use of intensity data to determine the three-dimensional one-electron density function is also discussed.

Consideration is given to the possibility of determining the momentum density distribution of atoms and molecules by measurement of the inelastically scattered electrons at large scattering angles from ionising collisions. That is followed by a section on high resolution, high energy impact spectroscopy with a review of the experimental results obtained. Finally, the book describes in detail the various experimental techniques used, with separate chapters on electron gun design, velocity analysers, electron detection devices, and atomic and molecular jets.

Most of the material presented is readily available elsewhere but it is useful to have the information collected in a single volume. Being completely self-contained, the book provides an appropriate balance between theory, experimental methods and results. **M. J. Capers**

MANTLE
FLUID OUTER CORE
INNER CORE
BASE OF CRUSTAL LAYERS
OUTER SURFACE

THE EARTH'S DENSITY
K. E. BULLEN
May 1975: 448 pages: hardback:
412 10860 7: £12.90
This definitive *magnum opus* draws together the widespread strands of evidence that have led to the present knowledge of the distribution of density throughout the earth and the planets. The book is an authoritative source of information on a topic which bears on practically all current solid-Earth geophysical research. Recent developments of particular relevance to geophysical mineral exploration and an understanding of the planets are treated in considerable detail.

Two New Outline Studies in Biology
THE SELECTIVITY OF DRUGS
ADRIEN ALBERT
May 1975: 64 pages: paperback:
412 13090 4: £1.30
This book presents an overall picture of the physical basis of drug action based on three principles – favourable differences in distribution, favourable differences in biochemistry and favourable differences in cell structure – and shows how these principles control selectivity.

BIOMECHANICS
R. McNEILL ALEXANDER
May 1975: 64 pages: paperback:
412 13080 7: £1.30
This book presents a broad consideration of the application of mechanics to the study of organisms. Descriptions of biological materials, animal locomotion, human mechanics, plant mechanics and cell mechanics are all included.

Leaflets on Earth Sciences books and on the Outline Studies in Biology Series are available from the publisher on request.

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SCIENCE COLLEGE

announcements

Awards

The Gravity Research Foundation (Gloucester, Massachussetts) has given the **Non-linear Graviton Award** to **Roger Penrose.**

The Chemical Society has announced the following honorary fellowships: **F. Lynen, L. Malatesta, Sir David Martin, CBE** and **G. Ourisson.**

F. Bronner has been awarded the **André Lichtwitz Prize** for his work on calcium transport.

R. P. Bodnaryk is the first winner of the **C. Gordon Hewittt Award** (offered annually by the Entomological Society of Canada for exceptional accomplishment related to the study of insects by a young scientist). The Society's **Gold Medal** has been awarded to **G. G. E. Scudder** (systematics, morphology and distribution of Lygaeidae).

Appointment

The Institution of Electrical Engineers has announced that its next president will be **R. J. Clayton.**

Miscellaneous

Lichtwitz prize. This prize is meant to reward French or foreign scientists who have distinguished themselves in research on calcium and phosphorus metabolism, whether in the clinical or more fundamental fields. Conditions of entry for the 1975 prize (applications by June 30) may be obtained from: Director of INSERM, c/o Dr L. Bonnot, 101 rue de Tolbiac, 75645 Paris CEDEX 13, France.

Pathology award. The Fondation de Physiopathologie Professeur Lucien Dautrebande will award a prize (500,000 belgian francs) during 1976 for work on human or animal clinical physiopathology, preferably having therapeutic implications. For further information: 35 chaussee de Liege (5200), Huy, Belgium (before December, 1975).

Endangered flora. Smithsonian Institution's *Report on Endangered and Threatened Plant Species of the United States* are available, on written request, from: Endangered Flora Project, Department of Botany, Smithsonian Institution, Washington, DC 20560.

Person to Person

Philadelphia – Sussex/London/Cambridge. Accommodation (centre of city, five bedrooms with usual American conveniences) for exchange with suitable study accommodation near University of Sussex, or in London, or easy access from London to Cambridge. Academic couple, three school-age daughters. Approximate dates: September 1, 1975 to May 15, 1976 (B. C. Griffith, Library of Science, Drexel University, Philadelphia, Pennsylvania 19104).

London accommodation. Wanted August (and possibly September) by visiting research biologist and family. Three bedroom flat or house within reasonable distance of University College, London. (Dr D. Whittingham, MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK; 01-387 7050, ext. 728).

London–Charleston. Accommodation required within reasonable distance of Medical University of South Carolina, Charleston from January, 1976 to January, 1977. Can exchange for modern 3 bedroomed house (central heating) in London (Dr R. M. Galbraith, Liver Unit, King's College Hospital, Denmark Hill, London SE5 9RS, UK).

Coleus blumei. Biologist interested in using this species for demonstration purposes and as object for aesthetic appreciation wishes to receive any information (reprints or correspondence) about colour and morphological mutations. Possible exchange of genetic materials (Dr R. E. Gross, 74 Tubingen, Albrechtstrasse 9, West Germany).

Griffith observer magazine. For January 1975. Copy wanted. If desired, would swop for *Observatory* equivalent (Lillian Thompson, Flat 2, 14 College Terrace, Brighton BN 2 2EE, Sussex, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

The Chemical Industry and the EEC. (A statement by the Trade Affairs Board of the Chemical Industries Association.) Pp. 4. (London: Chemical Industries Association, 1975.) [203

Flora of Tropical East Africa. Edited by R. M. Polhill. Bixaceae. By D. M. Bridson, Pp. 3. (London: Crown Agents for Oversea Governments and Administrations, 1975.) 15p. [213

Imperial Cancer Research Fund. Annual Report and Accounts 1974. Pp. 41. Scientific Report 1974. Pp. 165. (London: Imperial Cancer Research Fund, 1975.) (213

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1279: Second-order Linear Differential Equations with Two Turning Points. By F. W. J. Olver. Legendre Functions with Both Parameters Large. By F. W. J. Olver. Pp. 137–185. (London: The Royal Society, 1975.) UK £2.05; Overseas £2.15. [213

Energy Economy in Industry: 150 Fuel Saving Ideas. By Nigel Gwyther. Pp. 38. (Melton Mowbray: T.G.W. Industrial and Research Promotions, Ltd., 29 Dalby Road, 1975.) 80p. [243

Agricultural Development and Advisory Service. Science Arm—Annual Report 1973. Pp. xxxviii + 257. (London: HMSO, 1975.) £5.50 net. [253

Department of the Environment—Central Unit on Environmental Pollution. Pollution Paper No. 4: Controlling Pollution. Pp. iii + 31. (London: HMSO, 1975.) 42p net. [263

Archaeological Investigations in New Palace Yard 1972–74. Pp. 16. (London: Department of the Environment, 1975.) [273

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 270, No. 904: Natural Regeneration of *Tilia cordata* in Relation to Forest-Structure in the Forest of Biatowieza, Poland. By C. D. Pigott. Pp. 151–179. (London: Royal Society of London, 1975.) [273

Other countries

Energy Communications, Vol. 1, No. 1, 1975. Edited by R. N. Maddox. Pp. 1–110. Subscription rate for Vol. 1 (1975), 6 issues: $40 prepaid. Postage outside USA $6.30 per volume. (New York: Marcel Dekker, Inc., 1975.) [243

Institutt for Atomenergi, Kjeller, Norway. Kjeller Report 151: Multec Version 1.0. A Program for Multivariable Estimation and Control of Dynamic Systems—Users Manual. By Arild Ek and Erik Gran. Pp. 44. (Kjeler: Institutt for Atomenergi, 1975.) [243

Science Council of Canada. Report No. 23: Canada's Energy Opportunities. Pp. 135. $3.30. Summary. Pp. 28. (Ottawa: Information Canada, 1975.) [253

World Health Organization. Guide to Simple Sanitary Measures for the Control of Enteric Diseases. By S. Rajagopalan. With a section of Food Sanitation by M. A. Shiffman. Pp. 103. (Geneva: WHO; London: HMSO, 1974.) Sw.fr.32. [253

Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIV, No. 11: A Simultaneous Two-Channel System for Lunar Occultation Observations. By C. L. Morbey and J. M. Fletcher. Pp. 271–281. Vol. XIV, No. 12: Spectroscopic Observations of Stars in HII Regions. By David Crampton and W. A. Fisher. Pp. 283–304.(Victoria, BC: Dominion Astrophysical Observatory, 1974.) [253

World Health Organization. Food Additives Series No. 6: Toxicological Evaluation of some Food Colours, Enzymes, Flavour Enhancers, Thickening Agents, and Certain other Food Additives. Pp. 204. (Geneva: WHO; London: HMSO, 1975.) [273

Essai d'Explication Optique des Points Chauds de la Terre—Conséquences. Par Georges Dubourdieu. Pp. 55. (Meudon: College de France, Laboratoire de Géologie, 1975.) [273

Annual Report of the Zoological Institute, Faculty of Science, University of Tokyo, 1974. Pp.27. (Tokyo: The University, 1975.) [14

Glossary of Soil Science Terms. Pp.34. (Madison, Wisconsin: Soil Science Society of America, 677 South Segoe Road, 1975.) $1. [14

World Health Organization. Technical Report Series, No. 559: New Approaches in Health Statistics—Report of the Second International Conference of National Committees on Vital and Health Statistics. Pp.40. Sw.fr.5. International Histological Classification of Tumours No. 11: Histological Typing of Thyroid Tumours. By Chr. Hedinger, in collaboration with L. H. Sobin. Pp.28 + 46 figures. Sw.fr.28. (Geneva: WHO; London: HMSO, 1974.) [24

Guides and Criteria for Recreational Quality of Beaches and Coastal Waters. (Report on a Working Group convened by the Regional Office for Europe of the World Health Organization, Bilthoven, 28 October-1 November 1974.) Pp.iii + 31. (Copenhagen: Regional Office for Europe, World Health Organization, 1975.) [34

nature

June 5, 1975

As the Prince of Wales, so the Non-Proliferation Treaty

> Across the wires the electric message came:
> 'He is no better, he is much the same.'
>
> *On the Illness of the Prince of Wales, afterwards Edward VII*
>
> Attributed to Alfred Austin (1835–1913)

THE Geneva conference to review the working of the Non-Proliferation Treaty ended last weekend after four frustrating weeks in which relatively little apparent success was evident. Hopes that at last the pressure of a deadline might bring the ninety-odd nations to agreement on something substantial came to nothing. Nor even did there seem to be enough in prospect for the conference to adopt the recent European expedient of stopping the clock to allow more time for discussion. Not for the first time has it been noticeable that the only thing more sacred than an international nuclear treaty is an international airline reservation!

If there is disappointment that the review conference did little to make the treaty look more attractive to the nations still outside its orbit (and it must be said that none of the potentially nuclear non-signatories is likely to be lured into the fold in the foreseeable future), this disappointment must be tempered with an element of relief that the participants did manage to stick together and that there were no defections from the treaty.

It was expected that the nuclear powers, particularly the superpowers, would have an uncomfortable month, and much of the first week or so was taken up with trying to spot who was going to emerge as the standard-bearer of the non-aligned and scourge of the nuclear powers. Eventually it was Mexico which assumed this role and took the strongest line, but the hands of the non-aligned were tied by the failure of many of the signatories among the developing countries to appear at the conference. Thus the two-thirds majority which can so often be mustered in United Nations circles against the developed countries was not easily available. Even so, Mexico's mark was firmly left on such issues as the continuing need to negotiate a comprehensive test ban and the possibility of declarations on non-first-use and non-use of nuclear weapons against non-nuclear powers.

The activities of Mrs Thorsonn, the Swedish president of the meeting, came in for much praise. She delayed her intervention with a draft document until half way through the last week when there was growing despair that anything would emerge at all, particularly from Committee 1, charged with reviewing articles devoted to security assurances, measures for disarmament and nuclear-free zones, yet bogged down on procedural matters. Her draft found wide acceptance and eventually led to a consensus statement accompanied, not surprisingly, by a number of reservations from individual nations.

The nuclear powers could have good cause to feel pleased that they had emerged relatively unscathed from their time in the dock (they did have the advantage, one observer noted, of acting simultaneously as judge and jury).

Not so pleased were one or two other countries. Switzerland, Yugoslavia and Romania all made noises about being surrounded by countries armed with tactical nuclear weapons. With good cause did they fear that in time of crisis they might be trampled on without the superpowers caring too much. The most likely to take this complaint further is Yugoslavia and judging by the performance of the last week or so, the possibility of Yugoslavia's withdrawal from the treaty has seriously to be reckoned with. The pioneering work of India in finding a justification for nuclear development through the peaceful explosions route could handily be used by several nations in danger of being overrun.

There are, nevertheless, several positive things to have emerged from Geneva. The cause of international control both of nuclear fuel reprocessing centres and of peaceful nuclear explosion facilities has advanced considerably as a result of the conference. The concept of nuclear-free zones has been extensively aired, even if for the present only Africa seems likely to be a possible candidate for joining Latin America in this category. And there is to be a further review conference in 1980, preceded by an examination in two years' time at the General Assembly of the United Nations of progress in fulfilling the objectives of the treaty.

If this sounds like modesty in the extreme in advancing the cause of arms control, it is, and many will go away fearing that the treaty will only continue to hold together as long as it places no serious demands on its signatories. Perhaps the treaty is too big in its sweep to be able to survive indefinitely. The real paths of progress in the next few years may be back in more detailed fields—regional treaties, restraints on testing and controls on the transfer of material. ☐

European space comes of age

The European Space Convention has now been formally signed, and the senior staff of the new European Space Agency appointed. Angela Croome reports on how things are now likely to shape up.

SPACE collaboration was one of the first activities to bring together the countries of western Europe in a common enterprise which needed to reach a critical size that none of the partners could afford on their own. That was in the late 1950s, a long time ago. In the event, two organisations were set up, separating ends and means — the European Launcher Development Organisation (ELDO) and the European Space Research Organisation (ESRO); this was a bad beginning and a source of subsequent confusion and discord. There were always critics who urged that launching and experimentation should be kept together and that there should be a single organisation on the lines of NASA. And the mismatch in structure between the two organisations added strength to this argument. Eventually, the progressive escalation of cost and the regression in the practical value of the launcher element, together with rumblings over resignation from ELDO's initiating member, the UK, prompted the setting up in 1966 of a political council straddling both bodies (the European Space Conference (ESC) of ministers) and moves towards rationalisation and amalgamation. That was in 1966.

Rationalisation and amalgamation did not come until April 16, 1975 when the member countries gave political approval to the outstanding points of the European Space Convention, agreed in principle over a year before and formally signed at a meeting of plenipotentiaries in Paris last week. The delay of a year was caused both by having to wait for the outcome of France's review of her scientific spending and because of a lack of unanimity over the appointment of the new agency's director-general, which was linked to the disposal of other senior agency posts. Although the delay has not really mattered much in the long run the senior staff of ESRO, the caretaker body, have been grossly overworked and Europe's space plans have been made to look rather silly yet again. But ESRO can readily wear such nuisances. It has grown immeasurably in stature since the troubles of the mid-1960s, having pulled off several complex satellite projects and become an equal partner with the USA in a major applications project, the aeronautical satellite programme; it has also produced a convincing case for a key element in the forthcoming Spacelab–shuttle operation.

Gibson

The new budget was adopted and a revised programme put in hand last year in spite of the lack of formal agreement on the convention and the appointment of the director-general. The basic difference between the old ESRO arrangement and the new European Space Agency (ESA) is sharply reflected in the budget; the agency's is to be six times as large as ESRO's was. European collaboration in space became almost overnight "a very different animal" in the words of ESRO's then acting director-general and head of administration, Roy Gibson. In 1973 the budget was 40 million Accounting Units, each unit being roughly equivalent to $1 and in 1974 it was 240 MAU — so that from last summer (when ELDO had in reality already ceased to exist) ESRO, as ESA caretaker, was disposing of $1 million every working day. The justification for these increased sums was the adoption of a substantial applications satellite programme. This has been the central plank of the new agency, and by relying for legality on the existing fully ratified ESRO convention it could get going ahead of the birth of the agency itself. It is expected that ratification of the ESA Convention by the 10 member states will take at least two years, during which time ESRO's convention will continue to be the legal base of the agency's activity under a 'gentleman's agreement'.

Roy Gibson was unanimously adopted as director-general by the ESRO Council in March, as were five new directors who now fill posts that had lain empty for the past year. Inevitably the atmosphere and performance of the new agency will owe much to its first director-general.

What sort of man is Roy Gibson and how does his administrative style compare with that of his two predecessors at ESRO? The first ESRO Director-General, Professor (now Sir) Hermann Bondi, who is at present Chief Scientific Adviser to the Ministry of Defence, may best be described as the ESRO magician. He inherited major crises—and proved a stunning negotiator by simply sweeping recalcitrant ministers, hesitant officials and critical journalists off their feet by his persuasive tongue and instant arguments. He foresaw that ESRO's success would depend on applications satellites and he promoted them tirelessly (especially his particular baby, the aeronautical satellite project), thereby largely overturning ESRO's original remit, which rather puritanically restricted it to scientific research excluding the engineering aspects. He left ESRO healthy, even muscular, but a bit out of breath. The three years under the German scientific civil servant, Dr A. Hocker, was a complete contrast and perhaps gave the organisation the spell of quiet consolidation and development it needed after the Bondi whirlwind. Dr Hocker was so retiring that it was sometimes asked whether ESRO actually had a director-general. On the other hand he was scrupulously impartial and conscientious in the best civil service tradition. No national delegates were 'bamboozled', no 'deals' were made under the Hocker regime.

Roy Gibson has spent his working life (he is 50) in a variety of international administrative posts. He is no civil servant; he more resembles senior management in a multinational company. He is a glutton for work and long hours, and thrives on both. Pre-breakfast briefings and constant commuting by air are a regular pattern of his diary. He explains that the key to such a life is being able to drop off to sleep at any time—a talent he learned as a cipher officer in the Army during the Second World War. Another asset is his proficiency in several languages. As a Colonial officer in Malaya after the war he spoke Tamil; he subsequently picked up his French during 10 years of work on international safeguards for atomic energy with the UK Atomic Energy Authority and he speaks German at home with his half-Austrian half-Swedish wife.

What difference will the formal existence of the ESA make? Gibson puts first the fact that the agency is charged with rationalising the aerospace facilities of Europe and in particular "improving the world wide competitiveness of European industry"—in the words of the convention. An Industrial Policy Committee (IPC), which will take a long, hard look at the aerospace structure and performance of each member country, is already being formed. "It may need to tell some firms to drop out"—in the transponder/repeater field, for example—should there prove to be a glut of manufacturers. The IPC will hold its first meeting this summer and by the end of the year there should be some answers.

The other aspect of rationalisation is the pooling of national space projects. Gibson is much encouraged by the change in attitude that was apparent at the meeting of European space ministers in April. Everybody expressed themselves keen to "play the card of Europe", in the French minister's words. Previously, member countries upheld national and European interests at the same time. "The ESA objectives cannot be brought off without sacrifice", says Gibson, "and up till now 'sacrifice' is something someone else makes." An early test may be whether the Germans are willing to pool their direct-television broadcast satellite project.

Gibson is not a man for personal publicity though he seems to recognise the need for such an organisation as the ESA to have a face. He is rather clear on the management style that he intends to promote. He envisages a sharpening and streamlining of management responsibility, what he calls a much more integrated management approach. This can come about now that the full directorate (of nine) has been formed—five appointments had to await the decision on the director-general because of the distribution of jobs among member states. "People think it has all been cooked", says Gibson. "It has—but not in the sense that member countries have offloaded their duds on us. Today we have no 'diplomatic passengers' ". His integrated management philosophy he describes as "simplicity born of desperation". He practises a "wide discussion" of any problem among the directors and all other staff involved—an entirely free debate where everyone chips in but once the executive decision is taken he requires complete loyalty of execution. "I expect whole-hearted acceptance even if 49% didn't want that direction beforehand." And if not, then they can go and peddle their matches somewhere else. He admits he feels "very strongly" about a "team approach" but not in terms of vagueness—the term team is often used to shift responsibility on to someone else. "We simply cannot afford to be transformed into a woolly civil service-type outfit where people are carefully housed [for example] when they are not making a contribution."

How is any tendency of this kind to be prevented? To start with he considers work style of the ESRO–ESA type of operation is an asset. "In contrast to other organisations, for instance the OECD, there is a direct result—hardware." He explains that there is little paperwork involved and a rigid timetable. If things slip in preparing a space project everything goes awry. Consequently people at ESRO work very hard; they are enthusiasts and think nothing of working late into the night. "Most of our people are sold on space and the level of professional scrounging is extremely small." The way to keep things this way is above all to be extremely careful over recruiting. Gibson comments, however, that it is now eight years since people were recommended by member governments "to get them out of the way". The present standard of new staff is very high. The other aspect is tenure: "We need to steer a mid-course between security and bureaucracy". He sees the long-term contract as the key, and although the appointment of ESRO's director-generals was for three years, one senses that Gibson may be set for something more like a decade.

The stature of Europe-in-space is something that Gibson takes very seriously. He feels that the outside world does not appreciate Europe's capability. "No one doubts that Hughes, for instance, can do the whole thing" but it does not occur to them that western Europe as an entity can. The opportunity offered by the coincidence of the biennial Paris Air Show and the signing of the ESA Convention has not been missed, for example, and a three-day conference and demonstration of European capabilities has been put on for the benefit of key visitors, including some ministers. Non-ESRO projects such as Britain's defence communications link Skynet and the Franco-German regional satellite Symphonie as well as ground-stations were on view to show that Europe has the capability today to "do the whole thing". Part of this preoccupation is concerned with Spacelab, which Europe is building for the American Shuttle programme for the 1980s and to which it will contribute half the first scientific payload. Gibson feels there is a danger of making propaganda over Europe's first entrance into manned spaceflight, as almost equal partners with the USA. Proper use of the possibilities Spacelab offers is much more important and he is not convinced that what this means is any better understood in the USA than in Europe. Spacelab experiments will cost less and the transfer from drawing board to flight fulfilment will be much quicker than the present seven years. They need not be miniaturised nor automatic. Integrating this highly complex programme is a daunting problem which Gibson has spent a lot of time thinking over. Financial, institutional and payload use, as well as development, all require to be effectively tried together.

The success of Spacelab would undoubtedly be the ultimate justification of the new agency — and the climax of Roy Gibson's career as director-general. □

ESA looks ahead

In a few weeks the first satellite under the European Space Agency's new name will be launched.

This year's satellite, COS-B, will carry a single large experiment, capable of detecting gamma rays with energies exceeding 20 MeV. The next scientific satellite planned for launch (in 1976) is GEOS, which will continue the theme of several European satellites in carrying out integrated studies of the magnetosphere. The earlier HEOS II satellite was the first to measure the magnetospheric properties of the polar cusp region, and also discovered the plasma mantle; HEOS I (now in its seventh year) continues to provide information about related phenomena.

But it is the other satellites in ESA's present programme which really point the way ahead for science in space. This path is very much one of collaboration. On the International Ultraviolet Explorer satellite, also due for launch in 1976, ESA will be collaborating with both NASA and the United Kingdom (so the UK, as a member of ESA will be collaborating with itself!). This satellite should be accessible to any astronomer in much the same way that telescopes on Earth are used by many different members of the astronomical community.

More collaboration with NASA is involved in the International Sun Earth Explorer (ISEE) project, planned for 1977, which also follows the European tradition of investigating the interplanetary medium and Sun–Earth links. And ESA's biggest project, the Spacelab, ties the future of European space research even more closely to that of NASA; so the last satellite in the present programme, EXOSAT, is likely to be ESA's last independent scientific fling, for a good few years at least.

international news

Science to return to the White House

from Colin Norman, Washington

SOME leaders of the scientific community in the United States are happy, for a change, because President Ford has said that he will welcome them back into the inner corridors of power after more than two years of exile. Last week, Ford told a delegation of key Senators and Congressmen that he wants Congress to pass a bill to establish a small science policy office in the White House, headed by a Science Adviser to the President. The details remain to be worked out, however, and it will be several months before such an arrangement is in place.

Ford's decision follows months of agitation by many scientific organisations and by several Members of Congress, who were considerably irked when former President Nixon announced, in January 1973, that he no longer required the services of a full-time science adviser. Nixon dismantled the science advisory apparatus set up by Presidents Eisenhower and Kennedy, conferred the title of Science Adviser on the director of the National Science Foundation—a small agency out in the bureaucratic hinterlands—and severed the main formal link between the scientific community and the White House by scrapping the President's Science Advisory Committee (PSAC).

Those actions drew heavy criticism, chiefly on the grounds that the National Science Foundation is too far removed from the mainstream of government decision-making to play an effective role in shaping and coordinating policies. Another complaint was that Dr H. Guyford Stever, the Director of NSF, was forced to wear two hats, one as science adviser and the other as director of a research agency —a situation which could raise problems of a conflict of interests and which relegated science advice to a part-time activity.

In the face of such criticisms—which have come from no less a body than the National Academy of Sciences, and which also prompted the Senate to pass a bill last year to set up a three-member Council of Science and Technology Advisers in the White House—it is no surprise that Ford has agreed that his predecessor's actions should be reversed. But the nature of Ford's decision and the manner in which it was announced are a bit curious.

In effect, Ford has outlined roughly what he wants, and he has invited Congress to fill in the details and establish the science policy apparatus by legislation. According to James Cannon, Assistant to the President for Domestic Affairs, Ford "has opted for a single director (of a White House science policy office), someone with considerable ability and scientific standing. He will have . . . perhaps a staff of 10 to 15 assistants". Cannon added that the office would probably be equipped with a budget of between $1 and $1.5 million a year.

Stever

David

But, aside from acknowledging the fact that science policy will eventually return to the White House, the announcement leaves a host of unanswered questions. According to one source who attended the Congressional briefing, for example, no decision has yet been made on whether the science adviser's interests should include military matters; it is also unclear how the office would interact with other key policymaking bodies in the White House—especially the Domestic Council and the Office of Management and Budget—and left in the air is the question of what will become of the extensive science advisory structure that now exists in the National Science Foundation as a result of Nixon's 1973 reorganisation.

But perhaps the most curious aspect of the announcement is why Ford chose to ask Congress to establish the office by legislation. He could easily have created whatever apparatus he wants simply by issuing an executive order, just as President Kennedy did when he established the Office of Science and Technology in the White House in 1962 (and as President Nixon did when he scrapped it eleven years later). It could be, as one insider suggested, that it is "just Ford's style of government" — he simply wants to bring Congress in on the matter.

A less charitable interpretation, however, is that the Administration considers the matter to be of low priority, and Ford simply wanted to get it off his back. That interpretation is supported by the fact that Ford had asked Vice-President Rockefeller in December last year to make a study of arrangements for bringing science advice into Presidential decision-making but Rockefeller's report had been gathering dust for some time.

The decision to go to Congress on the matter has several important practical implications. To begin with, if a science policy office is established in the White House by legislation, no president would be able to abolish it without first getting express approval from Congress.

Of more immediate concern, however, is the fact that it will take Congress several months to pass a bill.

Abortion survey

PERHAPS the most comprehensive survey yet conducted of the effects of legalised abortion in the United States has just been published by the Institute of Medicine, an organisation which is part of the National Academy of Sciences. It offers strong evidence to support the oft-heard contention that liberalised abortion laws save many women's lives because they result in a larger proportion of abortions being conducted in proper medical surroundings, rather than in the offices of back-street practitioners or by self-induced means.

The basis of the survey was a comparison between data collected before and after an abrupt change in abortion laws in the United States, resulting from a ruling by the Supreme Court in January 1973. In a highly controversial decision, the court severely limited the ability of state governments to restrict abortions performed during the early stages of pregnancy.

While the number of legal abortions performed in the United States climbed by about 29,000 between 1972 and 1973, according to statistics collected by the federal government, the number of known abortion-related deaths declined from 128 in 1970 to 47 in 1973. Much of that decline, the committee suggests, can be attributed to a drop in the number of illegal abortions.

Although there are no reliable statistics on the numbers of illegal abortions being performed, the committee notes that a survey of data from the first year of New York's non-restrictive abortion law suggests that about 70% of the abortions obtained legally would otherwise have been obtained illegally.

The survey provides a wealth of information on the hazards associated with abortions performed at various stages of pregnancy.

- The risks of maternal death as a risk of abortions performed during the first trimester are low—about 1.7 deaths per 100,000 abortions. They are much lower than the risks associated with full-term delivery (14 deaths per 100,000 live vaginal deliveries) or with surgical removal of tonsils and adenoids (5 deaths per 100,000 operations).
- The risks associated with abortions performed during the second trimester are much higher—about 12.2 deaths per 100,000 abortions.
- Those risks, in fact, increase "not only from the first to second trimester, but also by each week of gestation", the committee suggests. At eight weeks or less, the mortality risk is less than 0.5 deaths per 100,000 abortions. Between 9 and 10 weeks, it is 1.7, at 11 to 12 weeks, it increases to 4.2 and by 16 to 20 weeks, it reaches more than 17 deaths per 100,000 abortions.

On the basis of those findings, the committee recommends that "laws, medical practices and educational programs should enable and encourage women who have chosen abortion to obtain it in the first three months of pregnancy".

As for the suggestion that liberalised abortion may become a substitute for contraception, the committee found the data to be limited, but they nevertheless "suggest that the introduction of non-restrictive abortion laws in the U.S. has not lead to any documented decline in demand for contraceptive services".

The committee notes that the 1973 Supreme Court decision has crystallised opposition to abortion in the United States, and that its report is likely to generate considerable controversy. In a classic case of understatement, for example, the committee accepts that since it does not discuss ethical issues or questions concerning the fetus, "this approach implies an ethical position with which some may disagree".

(The report, *Legalized Abortion and the Public Health*, No IoM 75–02, is available from the Institute of Medicine, 2101 Constitution Avenue NW, Washington DC 20418.)

October is generally regarded as the earliest that a bill could reach Ford's desk, but it is more likely to be early next year before Congress completes its deliberations. In that case, with the Presidential election looming in November and a good chance that a Democratic Administration will be elected, it will be difficult to persuade an able scientist to come to Washington for what could be a very short stint as science adviser.

Congress also has a chance to put its own stamp on the arrangement by specifying exactly what it should cover and how extensive it should be. Congress is likely, however, to give the President pretty much what he asks for, and allow him considerable leeway in deciding how the office should operate. For a White House policy office to have any effect, it must establish its own working relationships with other units in the Administration, and most important, the President must be able to work with it.

The fact that President Ford has acknowledged the need for a central science policy office is generally regarded as a welcome first step, however. As Dr Edward E. David jun., President Nixon's last science adviser, put it last week, "we're making progress, but it's a long road to get to something that will perform a useful function". ☐

Soviet scene

from Vera Rich

THE President of the Soviet Academy of Sciences, Mstislav V. Keldysh, has retired at the age of 64, on the grounds of ill-health. Originally a mathematician, whose researches included potential theory, the theory of confluent elliptic equations, and the theory of functions of a complex variable, Keldysh later became associated with the development of the aircraft industry, and his appointment as a Vice-President of the Academy of Sciences of the USSR in 1960 and his promotion to the presidency in 1961 was widely seen to be directly connected with the expansion of the Soviet space programme under Khrushchev. As President of the Academy, Keldysh was, perhaps inevitably, noted more for his extensive speeches on all possible scientific occasions than for any direct contributions to genuine research. During his presidency he received a number of foreign and Soviet honours, including membership of the Mongolian Academy of Sciences, the German "Academia Leopoldiana", honorary doctorates from the Universities of Wroclaw, Budapest, and New Delhi, and the Lenin Prize (1967). It has been announced that he is to be succeeded as President of the Academy by the relatively obscure figure of Academician Vladimir A. Kotel'nikov, who has been variously described as an astronomer and a radio-electronics engineer, four years older than his predecessor, although certain unofficial reports from Moscow suggest that this appointment was by no means a foregone conclusion, Academician Vladimir Kirillin, Vice-Chairman of the Council of Ministers of the USSR, was at one point a strong favourite for the post.

- The "Patrice Lumumba University for the Friendship of Peoples" in Moscow is preparing to celebrate its fifteenth anniversary. Founded originally as the "University for Friendship among Peoples" in 1960, it was intended, according to a statement by Mr Sofronov, Secretary of the "Solidarity Committee for the Countries of Asia and Africa" made during a visit to the Indonesian National University on February 21, 1960, to be an instrument for the training of a national intelligentsia for the Afro–Asian and Latin–American countries.

During the 15 years of its existence, the University has expanded from a student body of some 300 housed in a former military academy to some 7,500 students (5,000 from 89 emerging countries and 2,500 Soviet students) with a modern campus of functional design.

According to the official figures, the University has an impressive record in terms of specialists trained and alumni now occupying prominent posts as statesmen, public figures and highly placed academic and industrial personnel in their home countries. Yet the whole purpose of the University still seems in question. Its foundation was clearly not entirely for altruistic motives. As early as 1923, Lenin was looking to what is now called the Third World as the decisive factor in the growth of World Communism (*Pravda*, March 4, 1923). He saw the problem primarily in terms of the countries of the Far East, but by the late 1950s it was clear that his theories could with equal validity be applied to Africa and Latin America. Clearly, the chance of educating students from these post-colonial countries in Moscow, rather than leaving them to follow the traditional path to London or Paris was an opportunity not to be missed. The question still remains, however—why a separate university, rather than scholarships at the ordinary universities of the Soviet Union? Since the only language of instruction at the Lumumba University is Russian the problem cannot be one of linguistic convenience, since the students have to learn Russian anyway before beginning their studies.

Perhaps the answer lies in the selection techniques. The Russian students who attend the Lumumba University seem to be very carefully selected to act as unofficial "tutors" to their foreign co-students. And the foreign students themselves are selected very carefully by the Russian authorities. This might seem reasonable, since after all it is the Soviet Union which is financing the University, but a number of countries, particularly those of South-East Asia, maintain that the selection procedure constitutes a breach of their own educational regulations, and have refused exit permits to students selected for the Lumumba University.

Whatever the reason, the Lumumba University students seem to be well isolated from the main body of Russian student life, even from that of the nearby Lomonosov Moscow State University. From time to time, complaints are voiced by former students about their feeling of isolation, which, in spite of the official title of the University, smacks to them of racial prejudice.

● As far as the Lumumba University is concerned, the Soviet Union jealously guards its privilege of selecting students for enrolment, as host and financer of the scheme. It is well known, however, that this principle does not operate in reverse nor in the higher academic echelons. When it is a matter of conferences or visiting lectureships outside the Soviet Union, then the wishes of the hosts are disregarded, and the invited speakers frequently fail to arrive and are replaced by some irrelevant substitute.

This practice has, in the past, been frequently tolerated by the hosts in the name of cultural exchange, although the cultural benefit derived from the presence of the substitute speaker may be minimal. Occasionally, however, a firm stand is made. In April, 1975, Dr William McGill, President of Columbia University, announced that he would not "receive or deal with any visitors from the Soviet Union" until the Moscow Sinologist, Vitalii Rubin, a long-standing member of the illicit Sunday seminar for *refusnik* scientists, is granted a visa to take up his two-year-old invitation as a visiting lecturer at Columbia University. Dr McGill also said that he would "use every effort I can muster to persuade my colleagues, voluntarily, to withdraw . . . from all forms of exchange with the Soviet Union" until Rubin received the desired visa.

A hundred years ago

A STRANGE case of poisoning is reported from Stettin. A gentleman had bought a hat in a shop there, and, after having worn it for one or two days, was troubled with unbearable headache; at the same time little ulcers formed upon his forehead, his eyes were inflamed, and the whole of the upper part of his head was much swollen. It was evident that these symptoms were caused by the hat, and upon examination by a chemist it was found that the brown leather in the inside of the hat was coloured with a poisonous aniline dye. It appears that inflammation is unavoidable when this dye is in contact with any part of the skin.

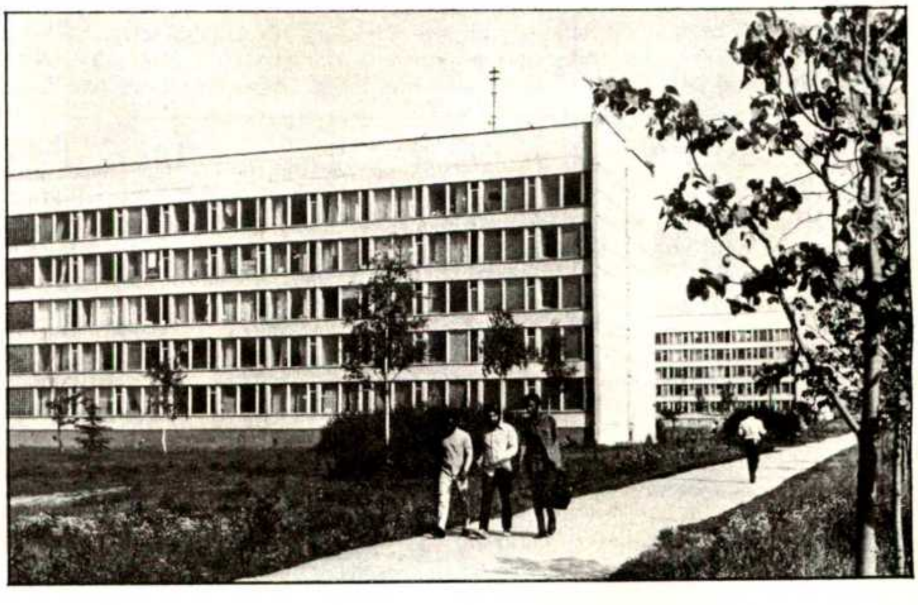
From Nature **12**, 116; June 10, 1875.

● Census taking of natural phenomena —from polar bears to ant-hills—is a regular feature of the Soviet academic scene. The latest "census" to be announced by the Novosti agency, that of sea ice, seems, in fact, to consist of the processing of existing data, gathered by Soviet Arctic and Antarctic expeditions and photographs and other information from weather satellites.

According to these results, the average annual volume of floating ice is 38,700 km^3, with an average daily accumulation/melt turnover of 37,000 km^3, a process which, apparently, takes place twice as fast in the southern hemisphere than in the northern.

It is stated that the ice-cover of all parts of the world ocean is completely renewed every 11 years. No connection has so far been suggested between this figure and the sunspot cycle. ☐

Patrice Lumumba University



Fission wastes under glass

from a Correspondent

DOWN among the vineyards freshly laden with grapes, and the arid scrublands of Provençal France, environmental pollution is entering a new phase of test and contestation. The Phénix fast-breeder reactor, which went critical in late summer 1973 and fully operational last year, has now produced a full year's electrical supply of its net annual output of 233 megawatts (a trifle under one-hundredth the current yield of the entire French national power grid). Situated on the Rhône River at Marcoule in an atomic energy installation inaugurated in 1953, Phénix once again reminds both the public and specialists of nuclear energy's number-one nag: what happens to the radioactive wastes?

To help answer the question, and capitalising on a Canadian idea, Phénix's technical managers—the Commissariat à l'Energie Atomique and Electricité de France—have spent the past six years exploiting their PIVER process of vitrification. The idea is to treat and then store in a minimal volume, irradiated fuel residues in a stable and inert form. PIVER is a chemical incorporation, rather than merely a physical packaging, involving the fusion of waste materials with glass containing aluminium compounds, after the elimination of nitrogen oxides and various volatile products of fission.

The waste-glass amalgam is cast into blocks of 100 kg each, poured from Inconel stainless steel pots 25 cm in diameter and 2.50 m tall. The blocks are then stored on the spot in concrete-sheathed, vertical tubes 10 m deep. The process is said to make the waste material as insoluble as is chemically and physically possible, confine the radiation trapped inside (a batch of 12 vitrified tons confines 4 million curies), and provide good thermal stability during the early cooling and first 10–15 years of storage. PIVER regularly handles 14 tonnes of irradiated waste, in two semi-weekly operations. The vitrified blocks are eventually removed from their storage shafts to a half-buried, more isolated site.

But is this enough? Even the Commissariat's Yves Sousselier, a chemist, asks, "Will there always be radioactive wastes?" Recently, a group of forty professors from the Collège de France circulated a petition to the Parisian government calling for a halt to all nuclear energy activity. But the government is proceeding with plans to start work later this year on Super-Phénix, a 1,200 megawatt breeder reactor to be built further upstream along the Rhône at Creys-Malville. □

KENNETH MELLANBY

Treble recorder

CHILDREN in Britain and other western countries are larger and heavier than were individuals of the same age a hundred, fifty, or even only thirty-five years ago. This is usually considered to be a sign of better nutrition, though reports of obesity—perhaps our commonest form of malnutrition—are disturbingly common. But even without the disadvantage of fatness, is the present type of feeding doing good or harm? We know that rats which have an apparently suboptimal diet when young live longer than their littermates given a more liberal diet. It will be interesting to find whether the total life span of the present generation of children is longer or shorter than that of those who, for instance, were reared during the slump in the nineteen thirties, or who had the advantage of war-time rations.

It is generally believed that as well as growing faster, children become sexually mature earlier than they did in previous times. There are many publications which are believed to prove this point. All are based on observations on girls, and describe the age of the menarche in different communities. It is said that this was quite young (eleven or twelve) in the Middle Ages, that it rose to sixteen or more years, at least in Scandinavia, in the nineteenth century, and that it has since fallen steadily to a figure similar to that found three or four hundred years ago.

These results obtained from girls, are not universally accepted, for some of the records of the phenomenon on which they are based, derived largely from orphanage records, are somewhat suspect. Also I have heard the topic discussed by a group of women scientists who are now in their nineties; they considered that they and their school friends, towards the end of the last century, were as precocious as the present generation. However, it was pointed out that they came from comfortable middle class homes, and that things might have been different among the ill-fed poor.

It always seems to me strange that there have been hardly any studies on boys, for here the onset of puberty could be so much more easily studied without the need for the sort of information which must have been hard to obtain among prudish Victorians. Some years ago I was involved in a pilot study of this subject, and I obtained the co-operation of Walter James, then editor of the *Times Educational Supplement*. I wished to find out whether there had been any change in the age at which boy's voices were observed to break. I thought it likely that we could find accurate records for this in the many choir schools attached to cathedrals and colleges in England.

The results of our study were, perhaps, surprising. We found that in the six schools investigated, there had been no significant change between the eighteen nineties and 1960. During the whole period boy's voices had broken, on an average, at a month or two after their thirteenth birthdays. Our preliminary figure suggested that there might have been a fall in this age by a few months in the seventy or so years covered by the investigation, but this was found to be a reflection of changes in the school system and not of the boy's physiology. At one time most choirboys remained at a choir school until the end of the session in which they ceased to sing treble; they now tend to be whisked off to a different school as soon as their voice breaks.

Our findings were briefly noted in Walter James' newspaper, and have received no notice from those concerned with this problem of allegedly earlier maturity. I think that the problem does warrant a much more exhaustive study. Discussion with school masters, particularly choir masters, suggests that there has indeed been little fall in the age of puberty of boys of all classes. This clearly has many sociological implications. But it does also mean that the female figures should be re-scrutinised. Both boys and girls have increased in height and weight in a parallel manner, it is strange that girls, apparently, should differ so much from boys in the change in the age of puberty.

● When I last referred to research contracts (May 1) I complained that they were often small and complicated to operate. In the majority of cases this is only too true. Unfortunately I gave the impression that the worst offenders might be found in the Department of Agriculture, Fisheries and Food. I am glad to be able to report that this is not so. Dr H. C. Pereira, the Chief Scientist, has been able to issue many sensible and simple contracts usually for very large sums. □

Asilomar conference on DNA recombinant molecules

At a meeting, held in California on February 24–27, an international group of scientists decided that strict controls should be placed on the use of an experimental technique which enables genes to be transplanted from one organism into another. This statement, drawn up by the organising committee for the conference is a summary of a report submitted to the Assembly of Life Sciences of the National Academy of Sciences and approved by its Executive Committee on May 20, 1975.*

This meeting was organised to review scientific progress in research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. Impressive scientific achievements have already been made in this field and these techniques have a remarkable potential for furthering our understanding of fundamental biochemical processes in pro- and eukaryotic cells. The use of recombinant DNA methodology promises to revolutionise the practice of molecular biology. Although there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant practical utility in the future.

Of particular concern to the participants at the meeting was the issue of whether the pause in certain aspects of research in this area, called for by the Committee on Recombinant DNA Molecules of the National Academy of Sciences, USA in the letter published in July, 1974, should end; and, if so, how the scientific work could be undertaken with minimal risks to workers in laboratories, to the public at large and to the animal and plant species sharing our ecosystems.

The new techniques, which permit combination of genetic information from very different organisms, place us in an area of biology with many unknowns. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult It is this ignorance that has compelled us to conclude that it would be wise to exercise considerable caution in performing this research. Nevertheless, the participants at the conference agreed that most of the work on construction of recombinant DNA molecules should proceed provided that appropriate safeguards, principally biological and physical barriers adequate to contain the newly created organisms, are employed. Moreover, the standards of protection should be greater at the beginning and modified as improvements in the methodology occur and assessments of the risks change. Furthermore, it was agreed that there are certain experiments in which the potential risks are of such a serious nature that they ought not to be done with presently available containment facilities. In the longer term serious problems may arise in the large scale application of this methodology in industry, medicine and agriculture. But it was also recognised that future research and experience may show that many of the potential biohazards are less serious and/or less probable than we now suspect.

Principles guiding the recommendations and conclusions

Although our assessments of the risks involved with each of the various lines of research on recombinant DNA molecules may differ, few, if any, believe that this methodology is free from any risk. Reasonable principles for dealing with these potential risks are: (1) that containment be made an essential consideration in the experimental design and, (2) that the effectiveness of the containment should match, as closely as possible, the estimated risk. Consequently, whatever scale of risks is agreed upon, there should be a commensurate scale of containment. Estimating the risks will be difficult and intuitive at first but this will improve as we acquire additional knowledge; at each stage we shall have to match the potential risk with an appropriate level of containment. Experiments requiring large scale operations would seem to be riskier than equivalent experiments done on a small scale and, therefore, require more stringent containment procedures. The use of cloning vehicles or vectors (plasmids, phages) and bacterial hosts with a restricted capacity to multiply outside of the laboratory would reduce the potential biohazard of a particular experiment. Thus, the ways in which potential biohazards and different levels of containment are matched may vary from time to time particularly as the containment technology is improved. The means for assessing and balancing risks with appropriate levels of containment will need to be re-examined from time to time. Hopefully, through both formal and informal channels of information within and between the nations of the world, the way in which potential biohazards and levels of containment are matched would be consistent.

Containment of potentially biohazardous agents can be achieved in several ways. The most significant contribution to limiting the spread of the recombinant DNAs, is the use of biological barriers. These barriers are of two types: (1) fastidious bacterial hosts unable to survive in natural environments, and (2) non-transmissible and equally fastidious vectors (plasmids, bacteriophages or other viruses) able to grow only in specified hosts. Physical containment, exemplified by the use of suitable hoods, or where applicable, limited access or negative pressure laboratories, provides an additional factor of safety Particularly important is strict adherence to good microbiological practices which, to a large measure can limit the escape of organisms from the experimental situation, and thereby increase the safety of the operation. Consequently, education and training of all personnel involved in the experiments is essential to the effectiveness of all containment measures. In practice these different means of containment will complement one another and documented substantial improvements in the ability to restrict the growth of bacterial hosts and vectors could permit modifications of the complementary physical containment requirements.

Stringent physical containment and rigorous laboratory procedures can reduce but not eliminate the possibility of spreading potentially hazardous agents Therefore, investigators relying upon "disarmed" hosts and vectors for additional safety must rigorously test the effectiveness of these agents before accepting their validity as biological barriers.

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Recommendations for matching types of containment with types of experiments

No classification of experiments as to risk and no set of containment procedures can anticipate all situations. Given our present uncertainties about the hazards, the parameters proposed here are broadly conceived and meant to provide provisional guidelines for investigators and agencies concerned with research on recombinant DNAs. However, each investigator bears a responsibility for determining whether, in his particular case, special circumstances warrant a higher level of containment than is suggested here.

Types of containment

(1) **Minimal risk:** This type of containment is intended for experiments in which the biohazards may be accurately assessed and are expected to be minimal. Such containment can be achieved by following the operating procedures recommended for clinical microbiological laboratories. Essential features of such facilities are no drinking, eating or smoking in the laboratory, wearing laboratory coats in the work area, the use of cotton-plugged pipettes or preferably mechanical pipetting devices and prompt disinfection of contaminated materials.

(2) **Low risk:** This level of containment is appropriate for experiments which generate novel biotypes but where the available information indicates that the recombinant DNA cannot alter appreciably the ecological behaviour of the recipient species, increase significantly its pathogenicity, or prevent effective treatment of any resulting infections. The key features of this containment (in addition to the minimal procedures mentioned above) are a prohibition on mouth pipetting, access limited to laboratory personnel, and the use of biological safety cabinets for procedures likely to produce aerosols (for example, blending and sonication). Though existing vectors may be used in conjunction with low risk procedures, safer vectors and hosts should be adopted as they become available.

(3) **Moderate risk:** Such containment facilities are intended for experiments in which there is a probability of generating an agent with a significant potential for pathogenicity or ecological disruption. The principal features of this level of containment, in addition to those of the two preceding classes, are that transfer operations should be carried out in biological safety cabinets (for example, laminar flow hoods), gloves should be worn during the handling of infectious materials, vacuum lines must be protected by filters and negative pressure should be maintained in the limited access laboratories. Moreover, experiments posing a moderate risk must be done only with vectors and hosts that have an appreciably impaired capacity to multiply outside of the laboratory

(4) **High risk:** This level of containment is intended for experiments in which the potential for ecological disruption or pathogenicity of the modified organism could be severe and thereby pose a serious biohazard to laboratory personnel or the public. The main features of this type of facility, which was designed to contain highly infectious microbiological agents, are its isolation from other areas by air locks, a negative pressure environment, a requirement for clothing changes and showers for entering personnel and laboratories fitted with treatment systems to inactivate or remove biological agents that may be contaminants in exhaust air, and liquid and solid wastes. All persons occupying these areas should wear protective laboratory clothing and shower at each exit from the containment facility. The handling of agents should be confined to biological safety cabinets in which the exhaust air is incinerated or passed through Hepa filters. High risk containment includes, in addition to the physical and procedural features described above, the use of rigorously tested vectors and hosts whose growth can be confined to the laboratory.

Types of experiments

Accurate estimates of the risks associated with different types of experiments are difficult to obtain because of our ignorance of the probability that the anticipated dangers will manifest themselves. Nevertheless, experiments involving the construction and propagation of recombinant DNA molecules using DNAs from (1) prokaryotes, bacteriophages and other plasmids, (2) animal viruses, and (3) eukaryotes have been characterised as minimal, low, moderate and high risks to guide investigators in their choice of the appropriate containment. These designations should be viewed as interim assignments which will need to be revised upward or downward in the light of future experience.

The recombinant DNA molecules themselves, as distinct from cells carrying them, may be infectious to bacteria or higher organisms. DNA preparations from these experiments, particularly in large quantities, should be chemically inactivated before disposal.

(1) **Prokaryotes, bacteriophages and bacterial plasmids:** Where the construction of recombinant DNA molecules and their propagation involves prokaryotic agents that are known to exchange genetic information naturally, the experiments can be performed in minimal risk containment facilities. Where such experiments pose a potential hazard, more stringent containment may be warranted.

Experiments involving the creation and propagation of recombinant DNA molecules from DNAs of species that ordinarily do not exchange genetic information, generate novel biotypes. Because such experiments may pose biohazards greater than those associated with the original organisms, they should be performed, at least, in low risk containment facilities. If the experiments involve either pathogenic organisms, or genetic determinants that may increase the pathogenicity of the recipient species, or if the transferred DNA can confer upon the recipient organisms new metabolic activities not native to these species and thereby modify its relationship with the environment, then moderate or high risk containment should be used.

Experiments extending the range of resistance of established human pathogens to therapeutically useful antibiotics or disinfectants should be undertaken only under moderate or high risk containment depending upon the virulence of the organism involved.

(2) **Animal viruses:** Experiments involving linkage of viral genomes or genome segments to prokaryotic vectors and their propagation in prokaryotic cells should be performed only with vector-host systems having demonstrably restricted growth capabilities outside the laboratory and with moderate risk containment facilities. Rigorously purified and characterised segments of non-oncogenic viral genomes or of the demonstrably non-transforming regions of oncogenic viral DNAs can be attached to presently existing vectors and propagated in moderate risk containment facilities; as safer vector-host systems become available such experiments may be performed in low risk facilities.

Experiments designed to introduce or propagate DNA from non-viral or other low risk agents in animal cells should use only low risk animal DNAs as vectors (for example, viral, mitochondrial) and manipulations should be confined to moderate risk containment facilities.

(3) **Eukaryotes:** The risks associated with joining random fragments of eukaryote DNA to prokaryotic DNA vectors and the propagation of these recombinant DNAs in prokaryotic hosts are the most difficult to assess.

A priori, the DNA from warm-blooded vertebrates is more likely to contain cryptic viral genomes potentially pathogenic for man than is the DNA from other eukaryotes. Consequently, attempts to clone segments of DNA from such animal and particularly primate genomes should be performed only with vector-host systems having demonstrably restricted growth capabilities outside the laboratory and in a moderate risk containment facility. Until cloned segments of warm-blooded vertebrate DNA are completely characterised, they should continue to be maintained in the most restricted vector-host system in moderate risk containment laboratories; when such cloned segments are characterised they may be propagated as suggested above for purified segments of virus genomes.

Unless the organism makes a product known to be dangerous (such as toxin, virus), recombinant DNAs from cold-blooded vertebrates and all other lower eukaryotes can be constructed and propagated with the safest vector-host system available in low risk containment facilities.

Purified DNA from any source that performs known functions and can be judged to be non-toxic, may be cloned with currently available vectors in low risk containment facilities. (Toxic here includes potentially oncogenic products or substances that might perturb normal metabolism if produced in an animal or plant by a resident microorganism.)

(4) **Experiments to be deferred:** There are feasible experiments which present such serious dangers that their performance should not be undertaken at this time with the currently available vector-host systems and the presently available containment capability. These include the cloning of recombinant DNAs derived from highly pathogenic organisms (that is, Class III, IV and V aetiologic agents as classified by the United States Department of Health, Education and Welfare), DNA containing toxin genes and large scale experiments (more than 10 litres of culture) using recombinant DNAs that are able to make products potentially harmful to man, animals or plants.

Implementation

In many countries steps are already being taken by national bodies to formulate codes of practice for the conduct of experiments with known or potential biohazard*† Until these are established, we urge individual scientists to use the proposals in this document as a guide In addition, there are some recommendations which could be immediately and directly implemented by the scientific community.

Development of safer vectors and hosts

An important and encouraging accomplishment of the meeting was the realisation that special bacteria and vectors which have a restricted capacity to multiply outside the laboratory can be constructed genetically, and that the use of these organisms could enhance the safety of recombinant DNA experiments by many orders of magnitude. Experiments along these lines are presently in progress and in the near future, variants of λ bacteriophage, non-transmissible plasmids and special strains of *E. coli* will become available. All of these vectors could reduce the potential biohazards by very large factors and improve the methodology as well. Other vector-host systems, particularly modified strains of *Bacillus subtilis* and their relevant bacteriophages and plasmids, may also be useful for particular purposes. Quite possibly safe and suitable vectors may be found for eukaryotic hosts such as yeast and readily cultured plant and animal cells. There is likely to be a continuous development in this area and the participants at the meeting agreed that improved vector-host systems which reduce the biohazards of recombinant DNA research will be made freely available to all interested investigators.

Laboratory procedures

It is the clear responsibility of the principal investigator to inform the staff of the laboratory of the potential hazards of such experiments, before they are initiated. Free and open discussion is necessary so that each individual participating in the experiment fully understands the nature of the experiment and any risk that might be involved. All workers must be properly trained in the containment procedures that are designed to control the hazard, including emergency actions in the event of a hazard. It is also recommended that appropriate health surveillance of all personnel, including serological monitoring, be conducted periodically.

Education and reassessment

Research in this area will develop very quickly and the methods will be applied to many different biological problems. At any given time it is impossible to foresee the entire range of all potential experiments and make judgments on them. Therefore, it is essential to undertake a continuing reassessment of the problems in the light of new scientific knowledge. This could be achieved by a series of annual workshops and meetings, some of which should be at the international level There should also be courses to train individuals in the relevant methods since it is likely that the work will be taken up by laboratories which may not have had extensive experience in this area. High priority should also be given to research that could improve and evaluate the containment effectiveness of new and existing vector-host systems.

New knowledge

This document represents our first assessment of the potential biohazards in the light of current knowledge. However, little is known about the survival of laboratory strains of bacteria and bacteriophages in different ecological niches in the outside world. Even less is known about whether recombinant DNA molecules will enhance or depress the survival of their vectors and hosts in nature. These questions are fundamental to the testing of any new organism that may be constructed Research in this area needs to be undertaken and should be given high priority. In general, however, molecular biologists who may construct DNA recombinant molecules do not undertake these experiments and it will be necessary to facilitate collaborative research between them and groups skilled in the study of bacterial infection or ecological microbiology. Work should also be undertaken which would enable us to monitor the escape or dissemination of cloning vehicles and their hosts

Nothing is known about the potential infectivity in higher organisms of phages or bacteria containing segments of eukaryotic DNA and very little about the infectivity of the DNA molecules themselves. Genetic transformation of bacteria does occur in animals suggesting that recombinant DNA molecules can retain their biological potency in this environment. There are many questions in this area, the answers to which are essential for our assessment of the biohazards of experiments with recombinant DNA molecules. It will be necessary to ensure that this work will be planned and carried out; and it will be particularly important to have this information before large scale application of the use of recombinant DNA molecules is attempted. □

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*Advisory Board for the Research Councils. *Report of the Working Party on the Experimental Manipulation of the Genetic Composition of Micro-Organisms* (*HMSO* Cmnd 5880, 1975).

†National Institutes of Health Recombinant DNA Molecule Program Advisory Committee.

news and views

Virological hazards in routine procedures

from Robin Weiss

DURING the past year there has been much discussion about the ethics and potential hazards of 'genetic engineering', particularly on the splicing together of unrelated DNA fragments with the aid of specific restriction enzymes. The public debate was set off at the 1973 Gordon Conference on Nucleic Acids (Singer and Soll, *Science,* **181,** 1114; 1973) and by Ziff's article on "Benefits and hazards of manipulating DNA" in the *New Scientist* (24 October, 1973), and led to the announcement by Berg *et al.* (*Science,* **185,** 332; 1974 and *Nature,* **250,** 175; 1974) of a voluntary and temporary 'moratorium' on the manufacturing of certain kinds of DNA recombinants. In Britain, this stimulated the setting up of a government Working Party under Lord Ashby's chairmanship on the Experimental Manipulation of the Genetic Composition of Microorganisms. The Ashby committee, whose report was published earlier this year (HMSO, Cmnd 5880), suggested that the use of these recombinants should not be prohibited provided that it were subject to rigorous control in the laboratory; it further recommended that the safeguards of traditional containment procedures should be reinforced by genetical devices to 'disarm' the microorganisms used and that studies should be made on the viability in the human intestine of laboratory strains of *E. coli,* such as K12 Progress on disarming was reported at the International Conference on Recombinant DNA Molecules at Asilomar, California, three months ago, and in this issue of *Nature,* Anderson (p. 502) and Smith (p. 500) report that *E. coli* K12 will survive and multiply for a few days in the human intestine but that viability is low, as is the transfer of plasmids to the indigenous *E. coli* population.

Recently the government Working Party, under the chairmanship of Sir George Godber, on the Laboratory Use of Dangerous Pathogens published its report (HMSO, Cmnd 6054), which was reviewed in last week's issue of *Nature.* The working party, set up following the fatal transmission of smallpox virus from an accidentally infected laboratory worker to two outside contacts, recommends much more stringent control than has hitherto been imposed over known human and animal pathogens.

Despite the recent concern about genetic engineering and laboratory use of dangerous pathogens, there has been little or no public discussion about the possibility of unknown pathogens that might be unwittingly generated to quite high titres during routine procedures of experimental biology. I am thinking here not only of the possibility that laboratory variants of viruses could be more harmful than the wild-type isolates from which they were derived, but also of the activation and selection of RNA tumour viruses, particularly in tissue transplants in immunodeficient recipients and in somatic cell hybrids.

Xenografts and xenotropic viruses

When McAllister *et al.* (*Nature new Biol.,* **235,** 3; 1972) implanted cells of a human sarcoma line into the brain of a foetal cat and subsequently re-established the tumour in culture, the cells were found to be chronic producers of a C-type virus, named RD114. At first this virus was thought to be of human origin but later several laboratories showed that it represented an endogenous feline virus, unrelated to the better known, horizontally-transmitted feline leukaemia viruses. The RD114 virus grows to high titres in human cells, but feline cells do not support its replication. In a similar study, Todaro *et al.* (*Proc. natn. Acad. Sci. U.S.A.,* **70,** 859; 1973) obtained a murine C-type virus, AT-124, from human sarcoma cells inoculated into mice treated with anti-thymocyte serum. This virus also grows selectively in human cells but not in mouse cells. New Zealand Black mice spontaneously produce a similar virus which has been implicated in the auto-immune disease complex characteristic of this inbred strain (Levy, *Am J. clin. Path* . **62,** 258; 1974).

Levy has named these viruses, 'xenotropic', in contrast to the 'ecotropic' leukaemia viruses that can propagate infectiously in their host species of origin Like some of the ecotropic viruses, xenotropic viruses appear to be transmitted in the 'resistant', natural hosts as Mendelian traits, probably representing integrated, DNA proviral forms of the viral genome. When activated to form mature virus particles, these can only infect and replicate in host cells of certain foreign species The implication of these findings is obvious, in view of the expanding use of xenografts of human tumour tissue in immunosuppressed and nude, athymic mice, but it has largely been ignored Immunodeficient mice are routinely held in containment facilities for their own protection from infection, but human tumour cells subsequently explanted from such hosts might be handled without special precautions by laboratory workers who may be wholly unaware that the cells may be releasing C-type viruses infectious for human cells.

I am not aware of any studies to ascertain whether or not nude (*nu*) mice harbour xenotropic viruses, but this kind of virus is carried by many commonly used inbred strains, such as NIH-Swiss and BALB/c, and the *nu* gene can be cross-bred into any genetic background. It is possible that xenotropic virus is not activated in *nu* mice, though partial immune suppression as well as immune stimulation (graft-*versus*-host reactions and mixed lymphocyte reactions *in vitro*) appear to selectively activate such viruses (Scherr, Lieber and Todaro, *Cell,* **1,** 55; 1974). A study of the status of endogenous viruses in *nu* mouse strains should be carried out before they are used even more extensively than at present.

Many transplantable mouse tumours (Lieber *et al., Int. J. Cancer,* **15,** 555; 1975) and cell lines in culture chronically produce ecotropic, xenotropic or both kinds of murine C-type virus. We do not know whether xenotropic murine viruses induce malignant growth, autoimmune disease or other diseases in susceptible hosts, and this question also demands early investigation. Rats and marmosets should serve as suitable hosts. Murine xenotropic viruses may prove to be innocuous for man, for mice and men have long lived together without apparent harm, but ignorance does not spell safety.

Cell fusion

All the potential hazards of endogenous xenotropic viruses raised already in relation to xenografts also apply to the use of somatic cell hybridisation techniques, with the added hazard that inactivated Sendai virus, commonly used as an aid to cell fusion, can render cells susceptible to viruses to which they are normally resistant.

Neff and Enders (*Proc. Soc. exp. Biol. Med.*, **127**, 260; 1968) first showed that poliovirus, which does not infect hamster cells, will replicate in and kill hamster cells if introduced through alterations of the cell surface mediated by inactivated Sendai virus This technique has also been used in several laboratories to overcome the genetic resistance of cells to infection with avian RNA tumour viruses and to mediate the infection of virus particles defective for envelope glycoproteins. In cell fusion experiments, the use of inactivated Sendai virus may cause the spread of latent viruses to the alternate host cell type. Stocks of Sendai virus used for cell fusion are prepared from large pools of infected chicken eggs that commonly contain congenitally transmitted avian RNA tumour viruses. The rate of inactivation of avian tumour viruses by ultraviolet light is some 20-fold lower than that of paramyxoviruses like Sendai (Levinson and Rubin, *Virology*, **28**, 533; 1966). Thus ultraviolet-inactivated Sendai virus stock distributed for cell fusion studies may frequently contain incompletely inactivated avian tumour virus, as I reported some years ago (*J. gen. Virol.*, **5**, 511; 1969). The avian tumour virus would not naturally penetrate mammalian cells but it will with the aid of inactivated Sendai virus; once introduced into a mammalian cell, the provirus formed may persist indefinitely in the cell's progeny as an inapparent infection (Boettiger, *Cell*, **3**, 71; 1974). β-Propiolactone rapidly inactivates RNA tumour viruses as efficiently as it inactivates Sendai virus, but this reagent is itself a dangerous and volatile carcinogen, and it must be handled with considerable caution.

Many of the cell types commonly used in cell fusion studies, L cells and murine lymphoma cells, spontaneously release C-type RNA viruses. Some of these viruses may be xenotropic, and xenotropic viruses may be activated in heterokaryons or hybrids, especially when 5-bromodeoxyuridine is used in the selection technique. Two recent papers on the expression of xenotropic viruses in interspecific hybrids between mouse and rat (Scolnick and Parks, *Virology*, **59**, 168; 1974) and between mouse and man (Gazdar, Russel and Minna, *Proc. natn. Acad. Sci. U S.A.*, **71**, 2642; 1974) suggest that the murine xenotropic virus is repressed in the hybrid cells. But, the mouse–rat hybrids were permissive for the replication of a murine ecotropic virus and for simian (woolly monkey) sarcoma virus, although the parent rat and mouse cells respectively were not susceptible to infection with these viruses. Therefore it should by no means be concluded that interspecific hybrids between resistant and susceptible species are resistant to virus replication Indeed, cell fusion was used several years ago to illustrate the rescue of Rous sarcoma virus (Svoboda, Machala and Hlozanek, *Folia Biol.*, **13**, 155; 1967) and SV40 (Watkins and Dulbecco, *Proc. natn. Acad. Sci U.S.A.*, **58**, 1396; 1967) from cells containing but not replicating the virus.

On mixed infection, RNA tumour viruses undergo high frequency recombination between host range and other markers (Vogt, *Virology*, **46**, 947; 1974; Kawai and Hanafusa, *Virology*, **49**, 37; 1972) and recombination takes place between endogenous and exogenous viruses too (Weiss, Mason and Vogt, *Virology*, **52**, 535, 1973) New and hazardous recombinants could arise in interspecific hybrids Phenotypic mixing between viral envelope components is an even more widespread phenomenon, giving rise to transient viral forms that may have a vastly extended host range (see, for example, Huang, Palma, Hewlett and Roizman, *Nature*, **252**, 743; 1974, Weiss, Boettiger and Love, *Cold Spring Harbor Symp quant. Biol.*, **39**, 1974). Fusion of cells that may separately harbour different chronic or latent infections may thus give rise to unwanted hybrid viruses, and the laboratory worker may be unaware when this happens.

None of the points I have raised about heterospecific cell fusion or transplants constitutes a proven virological hazard, neither do those raised over the introduction of animal virus genes into bacterial plasmids or viruses. My aim is merely to show how complex the consequences of cell fusion can be. Ironically, the virological laboratories are probably the safest ones, because only in those will the appropriate tests for detection be carried out.

Infectious DNA from normal cells

Hill and Hillova (*Nature new Biol.*, **237**, 35; 1972) first demonstrated the rescue of proviral DNA of Rous sarcoma virus in mammalian cells by 'transfection' to permissive chicken cells This technique has now been proven effective for the rescue of xenotropic endogenous proviruses in chick cells (Cooper and Temin, *Cold Spring Harbor Symp. quant. Biol.*, **39**, 1974) and in mouse cells (Scolnick and Bumgarner, *J Virol.*, **15**, 1293, 1975). Simply by adsorbing DNA extracted from normal mouse tissues to human cells, the xenotropic viral genome might be incorporated and activated to replicate in the recipient cells. Thus normal cellular DNA of a much used experimental species constitutes a potential hazard without incorporation into a plasmid or bacteriophage.

Are virus mutants dangerous?

The Working Party on the Laboratory Use of Dangerous Pathogens referred to already has recommended a severe reduction in the experimental use of pathogenic viruses in the UK Laboratory viruses that have been favoured for molecular biology and genetics, such as Fowl Plague virus, Newcastle Disease virus and Vesicular Stomatitis virus are placed in the same category as Smallpox virus, Marburg virus and the Lassa fever agent, in requiring the most stringent controls possible, whereas the report makes no mention at all of tumour viruses. Clearly, this will seriously affect experimental animal virology in this country and it will be a difficult task to balance the restriction to scientific endeavour against the risk to economically important animals.

Here I wish to stress the element of risk to the health of the laboratory worker studying virus mutants and variants. We usually assume that virus mutants which are conditionally or non-conditionally defective in replication must be less pathogenic than their wild-type progenitors, but this may not invariably be the case. Defective polyoma virus in mice, for instance, is more oncogenic than the wild type (Defendi and Jensen, *Science*, **157**, 703; 1967). Huang and Baltimore (*Nature*, **226**, 325; 1970) first suggested that the defective, interfering (DI) particles that arise on high multiplicity passage of many animal viruses may play a role in limiting viral disease processes *in vivo*. Where unrestricted viral replication is lethal this may well be true, for Doyle and Holland (*Proc natn Acad. Sci. U.S.A.*, **70**, 2105; 1973) have shown that administration of purified, homologous DI particles will considerably prolong the survival of mice inoculated intracerebrally with Vesicular Stomatitis virus. Instead of dying within 2 to 3 days of infection, the mice suffered a progressive, neural paralysis, which the authors named 'slow', though the mice began to die by 8 days post-infection. In some cases, excess DI particles inoculated with small doses of non-defective virus gave rise to genuinely slow, persistent infections. Now in mice the alternatives seem to be acute viraemia and early death, and persistent infection leading to paralysis In man, however, the choice is more likely to be acute viraemia leading to recovery and immunity, and persistent infection leading to paralysis

Similar arguments apply to the pathogenesis of temperature-sensitive (*ts*) virus mutants Some *ts* mutants of reovirus (Fields, *New Engl. J. Med.*, **287**, 1026; 1972) and of measles virus (Haspel and Rapp, *Science*, **187**, 450; 1975) cause hydrocephalus and runting in mice whereas the wild types kill the host quickly. A *ts* mutant of Vesicular Stomatitis virus paralyses hamsters whereas the wild type causes acute death (Stanners and Goldberg, personal communication). These mutants may serve as valuable models of persistent neurological infections and slow virus disease in man But the experimental mutants themselves may be dangerous.

These views are not intended to raise a hue and cry over 'callous and wicked' experimental biologists and it is to be hoped that the potential dangers alluded to will prove to be spurious. Nevertheless, it would be irresponsible not to bring these possible hazards to the attention of laboratory workers engaged in the biological manipulations discussed. This was not done in the two government reports and therefore no further reason is required for doing so here.

Pattern formation at EMBO

from R. S. Edgar

The First Annual EMBO Symposium on Developmental Genetics was held at Hirschorn, West Germany, from April 30–May 3.

ONE of the speakers remarked that when molecular biologists enter the field of development they find themselves rapidly retreating into the 18th century. I took him to mean that they find themselves wrestling with issues such as preformation and epigenesis (in modern guise, of course).

Histones

The meeting opened innocently enough with talks by E. Bradbury (Portsmouth Polytechnic) and R Kornberg (MRC Laboratory of Molecular Biology, Cambridge) on the recent substantial advances made in understanding the role the histones play in determining the primary structure of the eukaryotic chromosome. Bradbury pointed out that the 120 Å repeat found for chromatin a number of years ago by Wilkins was due largely to the histones and could be best explained by a regular bead model with the DNA wrapped around a histone core Kornberg provided evidence through careful nuclease digestion, chemical cross-linking and electron microscopic studies that the basic repeat structure was a tetramer bead consisting of one each of the histones H2A, H2B, H3 and H4. This bead protects 200 base pairs of DNA from nuclease digestion. Between the beads up to 20 base pairs of DNA are accessible to nuclease attack. This simple structure was apparently missed by earlier workers because of inadequate preparation procedures. Histone H1 is apparently not one of the basic bead histones —but Bradbury described some experiments implicating H1 in higher order packing of the chromosomes (The packing ratio of the DNA in chromatin is about 7: 1. In a metaphase chromosome, however, it is at least an order of magnitude greater, but the explanation for this is not yet known)

Fifty years ago the nature of the gene was a lively question Although largely settled now for prokaryotic organisms, this question is still open for the eukaryotes. In *Drosophila* the available evidence points to one band of the salivary chromosome as delimiting the extent of a gene. But the organisation of DNA in terms of structural and regulatory elements within this region remains to be resolved. A. Chovnick (University of Connecticut) summarised the state of his studies on the structure of the *rosy* locus in *Drosophila,* which controls the structure of the enzyme xanthine dehydrogenase and determines eye colour. He found that mutations affecting the structure of this enzyme in terms of electrophoretic mobility changes and also other putative missense mutations (mutants manifesting intragenic complementation) all map within 5×10^{-3} map units. This region is estimated to cover 4,000 nucleotide pairs of DNA, not grossly out of line with the number needed to code for xanthine dehydrogenase. Surprisingly, all the mutants that fail to make detectable protein also seem to map within this region. One might expect that some such mutants would be affected in regulatory elements. But Chovnick also reported the recent finding of mutants with alterations in the amount of enzyme made and also in the pattern of enzyme formation at different stages in development. One such allelic difference which affects the amount of enzyme formed has been found to be *cis* acting (and thus a possible operator-like element) and maps 5×10^{-3} map units away from the left boundary of the cluster of structural mutations This result suggests the possibility that genetic analysis of regulatory elements in at least one gene of *Drosophila* is in the offing.

Although a number of the presentations at the meeting dealt with attempts to understand the molecular events that lead to the differentiated state in cells, the major focus of the meeting seemed to be on the earlier and more enigmatic aspects of development. How is pattern established in organisms and what is the nature of the determined state?

Compartments

Significant new information has been obtained about the mode of development of the adult structures from the imaginal disks of *Drosophila.* This work was described first by P. Lawrence (MRC Unit of Molecular Biology, Cambridge) and then by A Garcia-Bellido (CSIC, Madrid, Spain). The various organs in the fly develop from small groups of cells which form early in development and subsequently proliferate, mainly during pupation. It has been found that development proceeds by the successive creation of 'compartments' and of compartments within compartments. These compartments have precisely defined boundaries across which cells do not migrate, thus any compartment may be thought of as a collection of founding cells (not necessarily of common lineage) whose descendants form the structures appropriate to that compartment. Using genetic mosaic analysis to trace clonal pedigrees, Garcia-Bellido has shown that for the dorsometathoracic (wing) segments anterior and posterior compartments are formed before hatching. A wing sub-compartment is then formed during the first larval instar and dorsal and ventral wing sub-compartments are formed about the same time.

Although the successive formation of compartments and sub-compartments progressively delimits the fate of cells, development and differentiation within a compartment is for a time at least regulative. Mosaic analysis indicates no reproducible topographical patterns of clones within compartments and clones of rapidly growing cells (clones of wild-type cells in flies of *minute* genotype) can outgrow the *minute* cells and nearly fill a compartment and still generate a normal differentiated pattern for that compartment. It appears that the homeotic mutants of *Drosophila,* known for many years, affect the genes which control development within compartments. The mutation *engrailed,* for example, acts to convert the posterior wing compartments into a mirror-image of the anterior compartment. Apparently the wild-type allele of the *engrailed* gene affects cell recognition and sorting properties during the creation of the anterior and posterior compartments since in the mutant, but not the wild type, cell mixing, as shown by clonal analysis, occurs across the boundary A variety of such homeotic mutants are known and their properties fit well with the notion that these genes determine the fate of cells within compartments.

Insects have long been considered archetypal representatives of mosaic as opposed to regulative development. In insects the first cells that form in the blastoderm are thought each to be a progenitor of a specific and different part of the adult structures In contrast, the vertebrate egg is considered highly regulative since the whole organism may be reconstituted from any single descendent cell, at least through the first several divisions of the egg. That the distinction between mosaic and regulative development has been overdrawn was argued by K. Sander (Albert-Ludwig's-Universität, Freiburg). He presented experiments which demonstrated convincingly that in insects the early developmental stages are to a degree still regulative. In the leaf hopper, if material from the posterior pole of the egg is moved anteriorly the fate of cells forming in adjacent regions of the blastoderm can be altered. When the eggs of blowflies are pinched by ligation the fate of cells adjacent to the ligated region is altered. If the egg is closed off in the middle, the anterior portion will generate only the anterior segments 1, 2, 3 and 4 while the posterior portion forms only segments 7, 8, 9 and 10. The central segments do not form. However, asymmetrical ligations can generate those segments (for example, 1, 2, 3 and 6, 7, 8, 9 and 10) These results are most readily interpreted by assuming that an inducer gradient exists within the egg which is re-established after ligation and that the fate of cells in the central region is determined by the re-established gradients. Sander argued that this prepattern formation which gives rise to the determined blastoderm occurs late in the primitive insects and with evolution is projected back into the oocyte giving rise to the so-called mosaic developmental pattern where each cell that arises in the young embryo seems to have a defined fate.

Pattern formation

P. Sengal (University of Grenoble, France) summarised his elegant studies on pattern formation in the skin of amniotes. This was one of the few presentations at the meeting dealing with vertebrate development. Whereas the epidermis is the sole participant in forming such major surface features as hair, feathers and scales, the dermis plays a crucial role in determining the topographical pattern of these elements. Sengal was able to demonstrate the role these two tissues play in differentiation by culturing together epidermis and dermis from different sources (mouse, chick, duck and lizard) on chick allantoic membrane. In all cases the epidermis initiated development of structures appropriate to its ancestry independent of the origin of the underlying dermis but the pattern of epidermally-derived elements within the epidermis seemed to be controlled by the dermis. Sengal found that dermis from the mouse upper lip (which normally forms large and small hairs) induced the formation of large and small placoids in lizard epidermis and large and small feather follicles in chick epidermis, whereas mouse dorsal epidermis (which normally forms small hairs only) induced a dense pattern of small scales or feathers in the appropriate epidermis Various combinations gave essentially the same answer—pattern information is transferred from the dermis to the epidermis even between mouse and lizard tissues. But although gross pattern information can apparently be transmitted between tissues derived from animals of different classes, the structures formed by the epidermis in these conditions resemble only the early stages of differentiation of these elements Apparently further specific instructions concerning the nature of the hair or

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Thermal environment of plants

from Peter D. Moore

THE annual report of the Department of Plant Biology of the Carnegie Institution can usually be relied upon to supply stimulating ideas and information in the field of plant ecophysiology and the recently issued report for 1973–74 is no exception. Of particular interest is a series of field and laboratory experiments relating to the influence of the thermal environment upon the growth and development of several plant species.

The field experiments are reported by Björkman *et al.* (*Carnegie Inst. Yb.*, **73**, 748; 1974) and concern transplantation into two experimental 'gardens', one in Death Valley, a desert site with high temperatures and low rainfall (less than 40 mm) and the other at Bodega Head on the coast of California, where conditions are cool and humid (annual rainfall is 750–1,000 mm). A total of 17 species were used in transplant experiments, including some species of cool, oceanic climates and some from desert environments, many belong to the family Chenopodiaceae, including species of both C_3 and C_4 type (see *Nature*, **252**, 438; 1974) In Death Valley all died during the summer if irrigation was withheld When supplied with water, however, eight of the species tested were capable of survival, but grew better between October and May than they did in full summer (June to September). One species, *Tidestromia oblongifolia*, not only survived at Death Valley when irrigated, but actually grew better in high summer than in the October to May period. All species except one of the cool, oceanic group failed to survive at Death Valley even when they were irrigated.

At the oceanic site, Bodega Head, irrigation had a far less marked effect and all species except one survived The exception, once again, was *T. oblongifolia* The experiments demonstrate the importance of temperature as a determinant of survival and growth under conditions of ample water supply in these species and they also indicate that adaptations which permit growth at one climatic extreme may preclude survival under less severe conditions.

Transplant experiments have certain disadvantages. Failure to survive at a site may be due to such pressures as predation or parasitism rather than to an adverse physical environment. Bjorkman and his colleagues have met this objection by conducting controlled environment chamber experiments on four species representative of the various responses observed in the field. The results obtained parallel the field observations. *T. oblongifolia* failed to sustain its growth under a 16 °C (day)/ 11 °C (night) thermal regime whereas it doubled its dry weight in three days when grown at 45 °C (day)/ 31 °C (night) These conditions reflect those often experienced at Bodega Head and Death Valley, respectively. The oceanic species again grew better under cooler conditions.

Growth differences between species and between temperature regimes cannot be explained simply in terms of differential translocation of photosynthate because growth analysis experiments showed a similar allocation of material to root, shoot and leaf in all species under both sets of conditions. So temperature must act directly upon metabolic processes, perhaps upon membrane or enzyme stability. The authors also draw attention to the fact that *Tidestromia* is a C_4 plant, as are some of the oceanic types, such as *Atriplex sabulosa*. The experiments thus confirm the association of the C_4 mechanism with adaptation to high temperature environments, but also show that some C_4 plants have growth optima at relatively low temperatures.

feathers is required but is not provided by the heterologous dermis Combinations of chick epidermis with duck dermis form proper feathers which resemble those of the duck in certain major features. Similarly mouse epidermis can be induced to produce hairs patterned by chick dermis if dermal cells from the palm (planta) of the mouse are interspersed between the mouse epidermis and chick dermis even though the planta dermis cannot by itself produce hair formation in mouse dermis Apparently a previous pattern message is required before specific instructions concerning the kind of structures to be formed is transmitted from the dermis to the epidermis. These results suggest a hierarchy of determinative events, events which for me were reminiscent of the behaviour of homeotic genes in *Drosophila* as described by Garcia-Bellido and by Lawrence.

Molecular and cellular basis

None of the systems described provide any insight as to the molecular or cellular basis of the patterning and determination events. The work presented by K. Illmensee (Institute for Cancer Research, Philadelphia) gave perhaps the closest look at what is involved at the cellular level. In the *Drosophila* embryo the four most posterior pole cells which are set aside during blastoderm formation proceed to form the gonad of the mature animal. Illmensee was able to show that this determination event is induced by cytoplasmic determinants localised in the posterior pole cytoplasm and that they appear at this specific location quite early in the development of the oocyte. This was demonstrated by withdrawing posterior pole cytoplasm from an egg (or oocyte), injecting it into another region of the embryo and letting development proceed to the blastoderm stage. The future fate of cells forming in the region of the injection was tested by transplanting them to the posterior portion of another embryo, suitably marked genetically, and testing the host animal for gonadal mosaicism and also by following the manipulations by electron microscopy. Characteristic cytoplasmic granules were found in the pole cytoplasm; later, similar granules were found in the nuclei of the presumptive gonadal cells But no causal connection between the granules and the determination event was asserted.

While these findings fit well the expectations of the Preformationists—that highly specific and irrevocable determination events occur early in insect development—the Epigeneticists might argue that the setting aside of the germplasm early in development is a special case and requires special mechanisms. Indeed, in all of the other systems described, whether insect or vertebrate, it appears that pattern formation is preceded by the creation of a pre-pattern of some sort which determines the future fate of cells and their descendants, not in terms of specific differentiation, but in terms of general pattern decisions such as anterior, posterior or large and small. Thus the specific fate of individual cells is progressively defined rather than predetermined.

This epigenetic view received little support from the analysis of the development and anatomy of the ventral nerve cord of the nematode *Caenorhabditis* presented by S. Brenner (MRC Laboratory of Molecular Biology, Cambridge). This nematode has a ventral nerve cord which serves to innervate the body muscles. The dorsal muscles are innervated by a dorsal nerve cord which consists of fibres derived from cells located in the ventral nerve cord. This cord is quite simple in basic plan, consisting of 58 neurones of only eight types as defined by their anatomy and by the connections they form with other cells.

The newly-hatched worm, however, has a ventral cord consisting of only 15 neurones, the other 43 are added to the cord shortly after through the multiplication, migration and differentiation of nine neuroblasts These events have been painstakingly followed in living animals. The pattern of division, migration and final fate of these cells is invariant and includes the death of certain cells in certain lineages.

These results are quite readily accounted for by a high degree of intracellular pre-programming of development and differentiation. Surgical experiments with a laser (in progress) may give more insight into the degree to which nematode development is regulative, if it is at all.

As Francis Crick (MRC Laboratory of Molecular Biology, Cambridge) remarked in his summary (to nobody's surprise) there is a very long way to go in explaining development in molecular and cellular terms. He also noted that except for the studies of homeotic mutants, the genetic analysis of development was curiously absent, and this in a meeting entitled Developmental Genetics.

Cropping natural animal populations

from Robert M. May

INTEREST in game ranching of natural animal populations as a food source goes back a long way. This interest is spurred by observations which suggest, for example, that in East Africa standing crops of conventional livestock (mostly cattle) run around 32,000 pounds per square mile on moderately well-managed grassland, and around 16,000 pounds per square mile on Masailand savanna, whereas wild game runs around 90,000 pounds per square mile on similar savanna (see, for example, Watt, *Ecology and Resource Management,* 70–72; McGraw-Hill, 1968).

Journals such as the *Journal of Wildlife Management* or the *East African Wildlife Journal* contain numerous studies of the detailed population dynamics of species that are possible candidates for cropping. Given these data, the problem is to find the management strategy that will give the optimum sustainable yield. Two lines of attack may be distinguished The first adopts some nonlinear or 'density dependent' model to describe the overall population dynamics (usually without age structure), and then uses econometric techniques to calculate the strategy which, under given assumptions, will maximise profits (for example, Clark, in a chapter in *Some Mathematical Problems in Biology,* Springer Verlag, 1974). The second constructs density independent 'actuarial tables' of age-specific survivorship and fecundity for the population, and then applies standard demographic techniques to find its intrinsic capacity for population growth and thus to estimate the sustainable yield (for example, Caughley and Birch, *J Wildl. Mgmt,* **35**, 658–663; 1971).

These approaches are useful first steps, but neither is conducive to finding the best age-specific cropping strategy. Beddington (*J. appl. Ecol.,* **11**, 915–924; 1974, and also Beddington and Taylor, *Biometrics,* **29**, 801–809; 1973) has recently proved a surprisingly general and elegant theorem which sheds light on this question. He shows that, for an age-structured population of constant size, the sustainable yield is maximised by the complete removal of one age class, effectively reducing the age of maximum longevity of the population, and a partial removal of a second age class. No other age class is touched. These two age classes depend, of course, on the demographic details; Beddington provides a simple algorithm for finding which two.

The basic theorem also applies if the crop to be maximised is biomass, or some sort of economic value, rather than simply numbers of animals. The analysis may be extended to find the optimum sex ratio, under various biological and economic assumptions It remains true that, for either sex, at most two age groups should be cropped: one partially and the other completely.

It is reassuring to note that these formal results accord with many traditional agricultural practices. For instance, in hill sheep farming a proportion of male and female lambs are removed, and at a certain age all ewes are removed; the ratio of males to females is kept constant, and at another age all males are removed.

The practical application of these strategic insights may be hindered by difficulties in determining the animals' ages. And there are situations where highly age-specific cropping would not be feasible, even were the ages known accurately. This may explain why Beddington's results were not derived earlier in the fisheries literature: in other respects, the theory of optimal management of fisheries seems much more developed than that of game ranching, for obvious economic reasons.

For a wide variety of theoretical assumptions as to survivorship and fecundity curves, Beddington's algorithm suggests that the maximum sustainable yield is obtained by cropping all the animals of an age somewhat beyond that of peak fecundity, along with a fraction of those in the youngest age class. The ensuing survivorship curves are strikingly similar to those found in many natural populations of higher animals (as reviewed, for example, by Caughley, *Ecology*, **47**, 906–918; 1966). There is currently a burgeoning literature which seeks to understand the evolution of life history strategies (for example, Goodman, *Am. Nat.*, **108**, 247–268; 1974; Schaffer, *Am Nat.*, **108**, 783–790, 1974; Southwood *et al.*, *Am. Nat*, **108**, 791–804, 1974), although Beddington's work was done with the practical aim of studying exogenous manipulation of animal populations, it is plausible to speculate that his nonlinear optimisation techniques may eventually be bent to a more general discussion of the way endogenous evolutionary forces shape survivorship curves.

Rotating superfluid film?

from P. V. E. McClintock

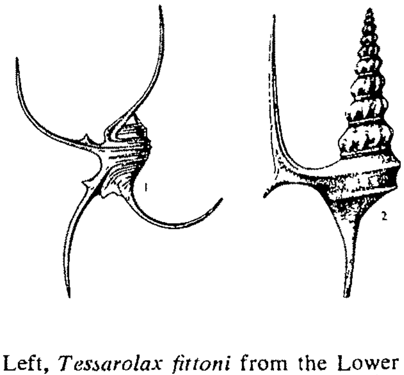
If one rotates a metal disk coated with a thin adsorbed film of superfluid helium, does the helium move with the disk or does it remain at rest? This is the intriguing question to which Vittoratos and Meincke of Toronto University have recently been addressing themselves and, in contrast with earlier workers, they seem to have been successful in establishing at least part of the answer (*Phys. Rev. Lett.*, **34**, 796, 1975).

Common sense seems at first sight to suggest that the helium film should move with the metal substrate with which it is in such intimate contact. On the other hand, if the helium is really superfluid then, by definition, there will be no frictional mechanism through which it can be brought into motion. There are, in addition, compelling theoretical reasons for believing that superfluid helium is actually incapable of rotating in a conventional way so that, even if an initially normal rotating film were cooled through the superfluid transition temperature at 2 K, one would not expect to end up with a rotating superfluid film.

The experimental chamber used at Toronto contained a capacitance cell formed from a pair of closely spaced aluminium plates 6 cm in diameter. It was anticipated that any rotation of the helium film adsorbed on the plates would result in a change of its thickness, thus leading to a change in capacitance because of the altered amount of dielectric between the plates. In fact, there were three separate capacitances each having the upper aluminium plate as a common element, the other elements being three small electrically isolated aluminium plugs mounted flush with the lower plate. One of these was positioned on the axis of rotation, and the others at radii of 1.4 and 2.0 cm from the axis. It was thus possible to measure the thickness of the film at these three positions with a resolution of about 2 Å. The films were prepared by condensing an appropriate quantity of gaseous helium into the cell so that, by carefully monitoring the capacitances, a film of any desired thickness up to about 300 Å could be established.

When the cell was rotated slowly it was found that the film was thinned to an equal extent at all measuring positions, thus demonstrating that the film itself was not rotating. if it had been, then the degree of thinning would have been different at different radial positions because of the centrifugal contribution to the potential energy of the adsorbed helium atoms (the overall uniform thinning indicating merely that there were regions within the rather complicated structure of the chamber, outside the capacitance cell, where the helium was in fact moving). When the angular velocity of the chamber reached a certain critical value, however, a difference in thickness developed abruptly between the film at 2 cm and that on the axis—suggesting that the film at 2 cm had come suddenly into some sort of rotational state.

Mesozoic fossils



Left, *Tessarolax fittoni* from the Lower Greensand, Isle of Wight; right, *Anchura carinata* from the Gault in Kent (From *British Mesozoic Fossils*, page 167; British Museum (Natural History), fifth edition, 1975.)

Now, in the case of bulk liquid helium it has been well established that the superfluid does not rotate in a conventional manner. Instead, the liquid in a rotating vessel becomes threaded with vortex lines, each consisting of a linear singularity or core around which the superfluid flows with a tangential velocity which is inversely proportional to the radial distance from the core. The net movement of the superfluid arising from the presence of the dense array of vortex lines generated within a rapidly rotating container does, in fact, approximate to that of normal solid-body rotation.

Vittoratos and Meincke have concluded from a detailed analysis of their experimental results that a somewhat similar picture is applicable to the helium film on a rotating substrate. They believe that the situation can be understood in terms of a critical radius r_c from the axis of rotation at which the tangential velocity of the substrate is equal to the critical velocity v_c for the onset of dissipation in a flowing film of the same thickness. Outside r_c there is a dense array of vortex lines which, by lying parallel to the rotational axis, permit an effective rotation of the superfluid at approximately the same angular velocity as the substrate, but inside r_c there are no vortices at all. On a rotating substrate, according to their proposed model, there should always be, around the axis of rotation, a tranquil vortex-free region of the film whose radius varies inversely with the angular velocity of the container.

This makes an interesting contrast with the situation in a rotating container of bulk liquid helium where it is a critical angular velocity which matters, and where the first vortex line is found on the axis of rotation. Another major difference between two- and three-dimensional rotation seems to be in the criteria for vortex formation. In bulk liquid the critical angular velocity may be computed by minimising the energy of the liquid as a whole, whereas, in the film, the first vortex is apparently formed when the tangential velocity of the fastest moving part of the substrate exceeds the linear critical velocity (ν_c) of the film. There is, as yet, no theoretical explanation of these differences.

Although many features remain to be investigated in further more precise experiments—it would, for example, be useful to determine accurately the extent to which the angular velocity of the film outside r_c lags behind that of the substrate—it is evident that this work has provided the first useful insight into the rotational behaviour of a two-dimensional superfluid.

Chemists and physicists in resonance

from J. A. S. Smith

When Ponce de Leon first landed on the Atlantic coast of Florida on April 3 1513, he is said to have been seeking "a never failing spring of such marvellous efficacy that when the water is drunk, it makes old people young again". The 105 participants at the 3rd International Symposium on Nuclear Quadrupole Resonance Spectroscopy who assembled in Tampa, Florida, just 462 years and 11 days later, were not perhaps expecting such a dramatic change in their lives, but in any event they almost certainly experienced a scientific rejuvenation.

ONE common theme of a number of papers was the investigation of static and dynamic critical phenomena at phase transitions. Quadrupole and magnetic resonance studies of frequencies, line-widths, and relaxation times now provide an important method of study, and the expected cusp (both maximum and minimum) in the relaxation rate, T_1^{-1}, as a function of temperature was shown to occur in a number of instances, such as ^{23}Na relaxation in $NaNO_2$ and ^{75}As relaxation in $NH_4H_2AsO_4$ (F Borsa and A. Rigamonti, University of Pavia). In related papers, R. L. Armstrong (University of Toronto) showed the importance of anharmonicity effects in the perovskite $CsPbCl_3$ by combining ^{35}Cl quadrupole resonance studies with neutron inelastic scattering spectroscopy, and H. Chihara (University of Osaka) presented further examples of displacive processes in the phase transitions observed in chloranil and ferroelectric $NH_4[H(ClCH_2COO)_2]$ The theme was continued in a number of contributed papers concerned with such diverse structures as $[CH_3NH_3]_2PtCl_6$ (D. Nakamura *et al.*, Nagoya University), $SbCl_3$ (R. J. C. Brown *et al.*, Queen's University, Kingston, Ontario), NaSH (K. R. Jeffrey, Queen's University), and $NaNO_2$ and $NaNbO_3$ (A. Avogadro *et al.*, University of Pavia). A related topic of much current interest is that of molecular motion and relaxation: A. Tzalmona (Hebrew University of Jerusalem) reviewed his applications of the sudden-jump approximation to such studies. Using ^{14}N quadrupole resonance, Y. Abe and his coworkers (Tokyo University of Education) demonstrated the presence of at least two molecular modes in ethylenediamine, in one of which the molecule twisted between two equivalent *gauche* configurations; T. A. Scott *et al.* (University of Florida, Gainesville) discussed the librations of the nitrogen molecule in both the pure solid and *p*-quinone clathrate; the motions of the PCl_3 group (I. G. Shaposhnikov *et al.*, Perm University, USSR) and $-BCl_3$ and $-CCl_3$ groups (S. Ardjomand and E. A. C. Lucken, University of Geneva) were also discussed in a number of molecules. E. Schempp (University of Pittsburgh) mentioned several crystals in which the acoustic phonon branches appear to dominate the negative temperature dependence of the frequencies at low temperatures, giving rise to a T^4 dependence.

The calculation of nuclear quadrupole interactions and electric field gradients in solids was reviewed by T P. Das (State University of New York, Albany); in non-cubic metals, pseudo-potential calculations have been applied in recent years with limited success to evaluate the contribution of the conduction electrons. An important conclusion of these calculations is that the electric field gradient depends on the whole electron distribution, not just on those electrons near the Fermi surface which dominate the Knight shift With solids such as Te, Se and S, tight-binding wave functions built out of atomic orbitals on the atoms need to be used, and even in non-cubic ionic crystals, recent work has shown the importance of allowing for the distortion of electronic orbitals by neighbouring ions through overlap and covalent bonding effects.

On the experimental side, the increasing importance of pulse techniques was evident in many papers. Among these, we may refer to the success of double resonance methods; R. Blinc (Institute J. Stefan, Ljubljana, Yugoslavia) reviewed very thoroughly these techniques and demonstrated that their sensitivity was now sufficient to detect ^{14}N quadrupole resonance of the peptide nitrogens in polyglycine. J. L. Ragle (University of Massachusetts, Amherst) discussed the elegant experimental methods by which he has detected ^{2}H quadrupole resonance with good resolution (<1 kHz) in many halogenated hydrocarbons, with a sensitivity sufficient to detect deuterons 5 to 6 Å away from the marker nucleus (for example the *β*-deuterons in the pyridine ring of $C_5D_5N.CDCl_3$). Other workers reported the double resonance detection of ^{55}Mn quadrupole resonance in carbonyls and ^{14}N quadrupole resonance in transition-metal glyoxime and bipyridyl complexes (T L. Brown *et al.*, University of Illinois, Urbana), and ^{27}Al quadrupole resonance in $AlBr_3$ (N Weiden and A. Weiss, Technische Hochschule, Darmstadt). Another new development is the revival of interest in high-pressure studies; in the alkylammonium halides (G. Jugie and J. A. S. Smith, University of London), chlorocyclophosphatrienes (W. H. Dagleish and A L. Porte, University of Glasgow) and trihalides of As and Sb (G. C. Gillies and R. J. C. Brown, Queen's University), the observed pressure shifts were related to changes in the intermolecular bonding.

In chemical applications of the technique, there is clearly a tendency to widen the range of nuclei that are studied. We have referred earlier to the deuteron, ^{27}Al, ^{55}Mn, and among others we may mention ^{51}V studies of V_2O_5 (V. A. Gubanov *et al.*, Ural Science Centre, Sverdlovsk) and the attempts now being made in several laboratories to study the structure of organometallic compounds by quadrupole resonance, for example ^{187}Re resonance in trimethylsilyl and trimethylgermanyl perrhenates (H. Schmidbaur *et al.*, Technische Universität, Munich), ^{59}Co resonance in dicobalt octacarbonyl and its alkyne derivatives (M. C. L. Gerry *et al.*, University of British Columbia, Vancouver), and ^{69,71}Ga, ^{115}In studies of group III organometallic compounds (T. B. Brill *et al*, University of Delaware, Newark).

The widening range of problems now being studied by nuclear quadrupole resonance and relaxation was very evident, which is perhaps a consequence of the close liaison which exists between chemists and physicists in this branch of radiofrequency spectroscopy. The Florida symposium did much to forward this collaboration.

High pressure living

from A. L. Rice

HYDROSTATIC pressure increases in the sea by one atmosphere for every 10 metres or so in depth, so that in the deepest parts of the oceans pressures well in excess of 1,000 atmospheres prevail and almost nine tenths of the ocean bed experience pressures of 100 atmospheres or more Until a little over a century ago these stupendous pressures, along with the other 'inhospitable' features of the deep-sea environment such as total darkness and low temperatures, were generally thought to preclude the existence of any life deeper than a few hundreds of metres. The great oceanographic voyages of the late nineteenth century dispelled this idea, for they demonstrated the existence of a rich fauna even at abyssal depths and stimulated an interest in the effects of high pressures on marine organisms, beginning with the classic works of Regnard and Certes in the 1880s and continuing, with a considerable upsurge in recent years, to the present day.

The instrumentation for this type of work is fraught with difficulties and for obvious technical reasons experiments on macroscopic organisms have, until fairly recently, been rather simple, usually being limited to the determination of lethal pressures for shallow-water animals. Nevertheless, a good deal of interesting information has been obtained. For one thing, although most of the shallow forms are killed by even short exposures to pressures much lower than those obtaining in the deep ocean trenches, many of them show a remarkable tolerance of pressures far outside their normal range of experience; common mussels (*Mytilus edulis*), for instance, may survive an hour's exposure to 800 atm. Second, the most pressure-tolerant shallow-water forms belong to just those animal groups which are most characteristic of the deep-sea fauna. Third, the degree of tolerance of individual species is dramatically affected by other environmental variables such as temperature, salinity and *p*H, and those animals which are most tolerant of variations in these factors are also most tolerant of high pressures (Flugel in *Marine Ecology*, vol. **1** (3) (edit. by O. Kinne) 1407–1450 (1972)).

For many years the potentially more illuminating experiments on the longer term effects of high but sub-lethal pressures were virtually impossible because of the difficulties of controlling such factors as the accumulation of metabolites and the depletion of oxygen in the small volumes of water in which the animals were necessarily confined. Modern experimental chambers which can be flushed under pressure have largely overcome this obstacle, but almost all of the available data are still from relatively shallow-living animals, so that the fascinating question of how deep-sea forms cope with their high ambient pressures remained unanswered.

Work on these deeper forms is just beginning and some of the most interesting results have been obtained from studies of the effects of pressure on the metabolism of oceanic pelagic crustaceans, many of which undergo diurnal vertical migrations through several hundreds of metres and therefore through several tens of atmospheres.

In the epipelagic forms tested, that is those which spend the night at, or close to, the surface, and migrate to depths of 200 or 300 m during the day, increased pressure within their normal range of experience has little or no effect on the respiratory rate. Low temperature, on the other hand, again within the normal environmental range, has a marked depressant effect on respiration, suggesting that by slowing their metabolism at their deep, cold, daytime levels these animals save energy by means of their diurnal migrations, despite the effort of swimming up and down (Teal and Carey, *Deep Sea Res.* **14**, 725–733; 1967).

In contrast, the mesopelagic migrants, spending the daytime at depths down to perhaps 1,000 m and rising to within 200 m or so of the surface at night, show an increased respiratory rate not only with increased temperature but also with increased pressure. Here the suggestion is that by a cunning balance of the two effects these animals manage to maintain a fairly constant level of metabolic activity throughout their migratory cycle, a necessity, it is argued, for predators who must be prepared to exploit a limited food supply wherever and whenever the opportunity presents itself (Teal, *Am. Zool.*, **11**, 571–576; 1971)

But these mesopelagic animals, despite their diurnal plunges, are nevertheless relatively shallow forms considering the total depth range of the oceans as a whole, and pressures not greatly beyond their normal range elicit general behavioural responses similar to those of their very shallow inshore relatives. Moderate pressure increases, usually less than about 200 atm., cause an increase in activity tending to become convulsive at the upper end of the pressure range. Activity is suppressed at slightly higher pressures, though the animals may recover upon decompression, but further compression, often around 400–500 atmospheres, causes tetany and even quite short exposures are often lethal.

Hardly any deeper-living species have been used in these kinds of experiments, but there is some evidence of an increased pressure tolerance, as one might expect, in these forms. The bathypelagic ostracod *Gigantocypris mulleri*, for example, with a normal depth range extending to 1,500 m, shows no change in its activity level at pressures up to 200 atm. and a somewhat greater tolerance of exposure to 500 atm. than most shallower animals (MacDonald, Gilchrist, and Teal, *J. mar. biol. Ass. U.K.*, **52**, 213–223; 1972).

In the latest contribution to the high pressure literature (*Deep Sea Res.*, **22**, 131–144; 1975) MacDonald and Teal have confirmed and extended some parts of these earlier results, but have demonstrated further complications in the general pressure story. Another bathypelagic species, this time the amphipod *Lanceola sayana*, showed broadly similar pressure responses to those of *Gigantocypris* whereas rapid compression caused convulsions in the mesopelagic decapods *Systellaspis debilis* and *Acanthephyra purpurea*, and immobilisation at 200 atm. or even less. Although these mesopelagic species had shown a definite enhancement of respiratory rate with increased pressure in the earlier work, no such relationship was found in the new longer term respiration experiments. The oxygen consumption rate was, however, increased by disturbance of any kind, including changing the water in the experimental chamber, emphasising the difficulty of interpreting present experimental results in terms of the animals' performance in the natural environment.

Pressure is clearly an important environmental variable in the sea; relatively shallow-water animals seem to make use of it in various ways while deeper living ones appear to avoid its consequences. There are many potentially fruitful lines of future research, but one of high priority must surely be to study the physiology of deep-sea animals collected and maintained at their usual environmental pressures instead of being committed, as now, to the rigours of a conventional net. It has been fashionable in recent years to blame the high temperatures of the near surface layers for the virtual impossibility of bringing up living animals from more than about 1.500 m. But considering the dramatic effects of high pressures on shallow-living organisms it is at least possible that exposing a deep-sea creature to ambient pressures tens or even hundreds of atmospheres less than its normal one doesn't do it much good, quite apart from the battering it must get in the net! There are already indications that some seemingly dead benthic animals may recover if they are quickly recompressed (Menzies, George and Paul, *Int. Rev. ges. Hydrobiol.*, **59**, (2), 187–197; 1974); under constant pressure they might not have 'died' in the first place.

The recovery of benthic animals under pressure presents special problems, but the technology to do so with planktonic forms has apparently been available for some time (MacDonald and Gilchrist, in *Barobiology and the experimental biology of the deep sea* (edit. by R. W. Brauer), 394–407 (1972)). Its use is long overdue and many biologists await the outcome with interest; the pressure is surely on!

review article

EPIC as a window on the forces of nature

Geoffrey Manning*

A proposal has been made to the Science Research Council for the construction of a new high energy physics accelerator called EPIC: electron positron intersecting complex. Because of recent experimental discoveries which have challenged earlier theoretical ideas, there is a general expectation that a major advance in our understanding of the basic forces of nature is imminent. EPIC, if constructed, will open up a new energy regime that will provide vital information to guide this advance.

ALL that is known about the laws of physics is based on observations over a range of distances from 10^{-13} to 10^{29} cm and for times between 10^{-24} and 10^{18} s. All of these observations—the whole of mankind's knowledge of the physical universe—can be explained in terms of the four basic interactions, gravitation, electromagnetic, weak and strong (Table 1). Gravitation is the weakest of the forces, its strength is 10^{-38} that of the strong interaction, we have an adequate theoretical understanding of it and it will not be discussed further here. The strong interaction, which is responsible for the binding of neutrons and protons within the atomic nucleus, has a range of $\sim 10^{-13}$ cm, is mediated by the exchange of particles of non-zero mass such as π mesons or K mesons. It is the strongest of the forces and we have no deep theoretical understanding of its behaviour. The electromagnetic interaction is responsible for the force between charged particles, its strength is 1/137 that of the strong force, its range is infinite and it is mediated by the exchange of photons—the zero mass quanta of the electromagnetic field. The remaining force—the weak interaction—is responsible for the decay of many, otherwise stable, particles—for example, for the decay of the neutron into a photon, electron and neutrino ($n \to p + e^- + \nu$). The neutrino is a neutral particle of zero mass that has no strong interaction and only feels the weak interaction which is 10^{-5} that of the strong interaction. Experimentally the weak interaction seems to have zero range and no particle has been discovered that could mediate the interaction if its range is non-zero.

Our current understanding

Our present understanding of the three interactions relevant to elementary particle physics is very varied. The theory of the electromagnetic interaction—quantum electrodynamics (QED)—is well understood. It is possible to calculate quantities with great precision and as yet no discrepancy with experimental observation is known even though checks have been made to the level of about 1 part in 10^8 in the case of the magnetic moment of the electron and the muon[1]. These high precision tests are sensitive to the detailed nature of the interaction at distances of $\gtrsim 10^{-14}$ cm—they test the perturbation expansion to high order. High energy, scattering experiments are necessary to test the theory at even smaller distances. The reaction $e^+ + e^- \to e^+ + e^-$ is dominated by the exchange of a single virtual photon and higher order terms—exchange of two photons for example—contribute only at the 1% level. The reaction has been studied[2] at 5 GeV in the centre of mass confirming QED to distances $\sim 10^{-15}$ cm. Tests to even shorter distances require higher energies.

The apparently zero range of the weak interaction is assumed by the Fermi theory, which represents it as a point interaction and is very successful in correlating a large body of data. It predicts that the cross section for the weak interaction should increase as the square of the centre-of-mass energy, resulting in an infinite cross section as the energy tends to infinity. This cannot be and the theory is clearly wrong. We must give up the assumption of a point interaction and thus must have a particle to carry the force over the non-zero range. Experimental searches for this "intermediate vector boson" have been unsuccessful and its mass is probably greater than 20 GeV. Figure 1 shows the cross section for neutrinos on protons as a function of neutrino energy and indicates the predicted cross section if an intermediate boson is assumed to exist. The assumed existence of a charged intermediate vector boson cures the problem of the increase in cross section with energy but the higher order corrections corresponding to the exchange of more than one such boson are infinite: the theory is divergent.

The past few years have brought a major theoretical advance. Several ideas have been developed which assume a fundamental

Table 1 Properties of the four basic forces in nature

Force	Form of interaction	Relative strength	Range	Mediator
Gravity	Acts between any particles having mass	$\sim 10^{-38}$	Infinite	Graviton (zero mass)
Electromagnetic	Acts between charged particles	$\sim 10^{-2}$	Infinite	Photon (zero mass)
Weak	Responsible for decay of otherwise stable particles, for example, $n \to p + e^- + \nu$	$\sim 10^{-5}$	Seems to be zero $< 10^{-15}$ cm	No suitable particle seen experimentally
Strong	Binding force between nucleons in nuclei	1	$\sim 10^{-13}$ cm	Many particles known, for example, π meson, k meson

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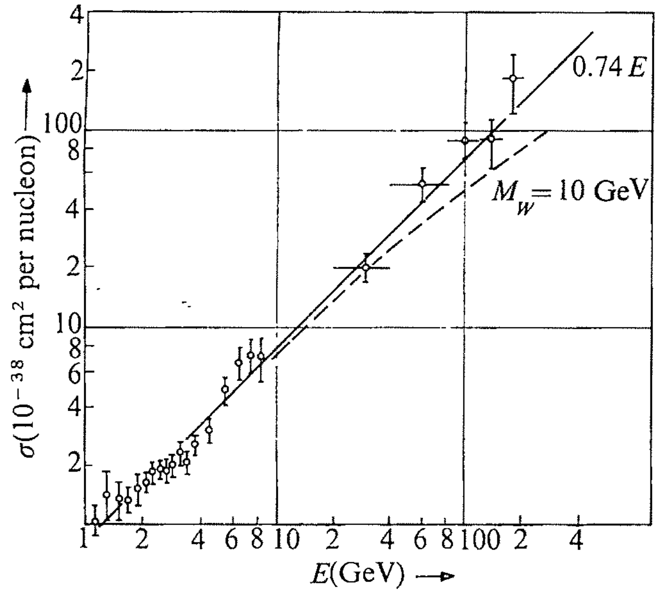


Fig. 1 Experimental results for the cross section of neutrinos on nucleons. The results are consistent with the cross section increasing linearly with energy—that is with a point interaction. The dashed line shows the cross section predicted on the assumption that the weak interaction is mediated by an "intermediate vector boson" of mass 10 GeV

unifying connection between the weak and electromagnetic interactions. Many of these theories require the existence of a neutral intermediate vector boson as well as the charged bosons. Experiments at CERN[3], later confirmed at FNAL[4], have shown conclusive evidence for the existence of neutral current contributions to neutrino interactions compatible with the predictions of the unified theories; but direct evidence for the existence of neutral or charged intermediate vector bosons has yet to be found and is unlikely until experiments can be made at higher energies.

The unification of two of the basic forces of nature is analogous to the great advance made by Clerk Maxwell in the nineteenth century when he combined the then separate theories of electric and magnetic phenomena within a single electromagnetic framework. This was the basis of our modern theory of electromagnetism and its ramifications were immense. The new ideas for unifying weak and electromagnetic interactions may be extendable to include the strong interaction and even gravitation—if so it is difficult to exaggerate its significance.

Particles that interact through the strong interaction are called hadrons. They can be divided into two main groups—baryons and mesons. Baryons are similar to the proton but all others are unstable and decay resulting eventually in a single proton in the final state, excess energy being carried off by the emission of electrons, neutrinos or gamma rays. Often the break-up involves a complicated chain of decays. This conservation of protons is recognised by allocating all baryons a quantum number, the baryonic charge, $B = 1$. As fas as we know this baryon quantum number is completely conserved in all reactions. There is a corresponding set of antibaryons ($B = -1$) each decaying and leaving a stable antiproton in the final state.

Mesons are strongly interacting particles with $B = 0$. They are all unstable and decay ultimately into electrons, neutrinos or gamma rays. If they are massive enough they can decay into a baryon–antibaryon pair as this conserves baryon number.

Throughout the past 10–20 years a great body of data on strong interactions has accumulated. The number of hadrons known has increased dramatically and is now several hundred. This glut was difficult to reconcile with the concept that they were elementary and a major advance was made when all known hadrons were classified in terms of six basic building blocks: three quarks and their corresponding antiquarks (Table 2). Each of these quarks is assumed to have $B = \frac{1}{3}$ and the antiquarks $B = -\frac{1}{3}$. Baryons are built of three quarks giving $B = 1$; antibaryons from three antiquarks; and mesons from one quark and one antiquark.

Table 2 Properties of quarks and antiquarks in three-quark model

		Charge	Baryon number (B)	Strangeness (S)	Spin	Parity
Quarks	u	$\frac{2}{3}$	$\frac{1}{3}$	0	$\frac{1}{2}$	+
	d	$-\frac{1}{3}$	$\frac{1}{3}$	0	$\frac{1}{2}$	+
	s	$-\frac{1}{3}$	$\frac{1}{3}$	-1	$\frac{1}{2}$	+
Antiquarks	$\bar{u}$	$-\frac{2}{3}$	$-\frac{1}{3}$	0	$\frac{1}{2}$	—
	$\bar{d}$	$\frac{1}{3}$	$-\frac{1}{3}$	0	$\frac{1}{2}$	—
	$\bar{s}$	$\frac{1}{3}$	$-\frac{1}{3}$	1	$\frac{1}{2}$	—

To account for other conserved properties discovered by experiments in strong interaction physics, quarks are given other quantum numbers including "strangeness". Strangeness is additive as electric charge and baryonic charge are additive and is conserved in the strong interaction although it is not conserved by the weak interaction.

The quark model not only classifies all known states but also gives approximate values for the masses, the decay rates, the magnetic moments, relationships between cross sections and so on. Its considerable success, however, is limited by several weaknesses, some theoretical but one major one of which is that experimental attempts to create and observe free quarks have failed. This could mean that quarks are very massive or that they are just a mathematical abstraction.

Further and more direct evidence for a substructure to the proton has been provided by experiments involving electron scattering. The behaviour of inelastic electron scattering from protons can be simply explained by assuming that a point-like electron is scattered from a point-like constituent charged part of the proton by the electromagnetic interaction. Similar and complementary evidence is obtained from neutrino scattering involving the weak interaction. These experiments not only directly demonstrate the existence of a substructure but they also measure the properties of the constituent parts. The results are consistent with the assumed properties of the quarks.

The recent discovery[5-7] of two new particles of mass 3.1 and 3.7 GeV with widths a factor of one thousand less than that normally expected, and a further state at 4.2 GeV (ref. 8) with a more normal width, has raised the question of yet another theoretical proposition: a fourth quark with a new quantum number, charm[9]. Previously discovered particles are constituted of the three quarks listed in Table 2 and the new states contain quarks with charm +1 and/or antiquarks with charm = −1.

Table 3 Properties of quarks and antiquarks in four-quark model

		Charge	Baryon number (B)	Strangeness (S)	Spin	Parity	Charm
Quarks	u	$\frac{2}{3}$	$\frac{1}{3}$	0	$\frac{1}{2}$	+	0
	d	$-\frac{1}{3}$	$\frac{1}{3}$	0	$\frac{1}{2}$	+	0
	s	$-\frac{1}{3}$	$\frac{1}{3}$	-1	$\frac{1}{2}$	+	0
	c	$\frac{2}{3}$	$\frac{1}{3}$	0	$\frac{1}{2}$	+	1
Antiquarks	$\bar{u}$	$-\frac{2}{3}$	$-\frac{1}{3}$	0	$\frac{1}{2}$	—	0
	$\bar{d}$	$\frac{1}{3}$	$-\frac{1}{3}$	0	$\frac{1}{2}$	—	0
	$\bar{s}$	$\frac{1}{3}$	$-\frac{1}{3}$	1	$\frac{1}{2}$	—	0
	$\bar{c}$	$-\frac{2}{3}$	$-\frac{1}{3}$	0	$\frac{1}{2}$	—	-1

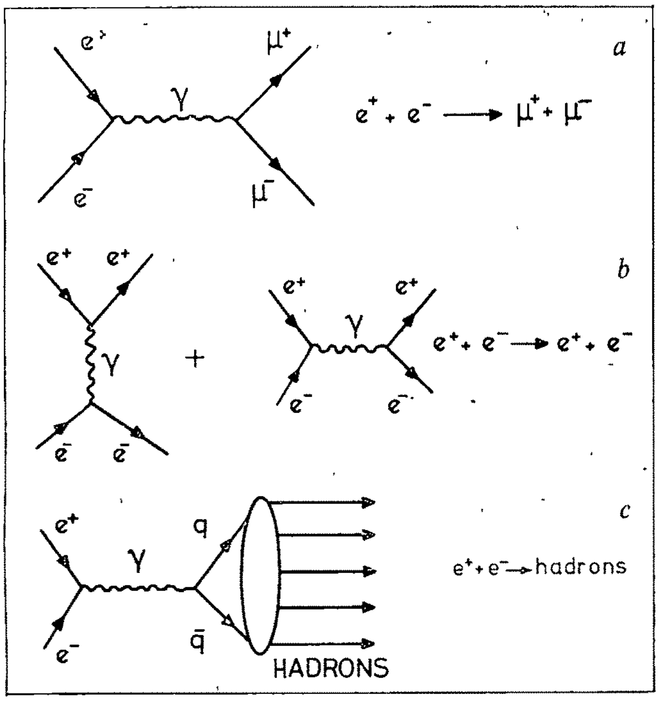

e^+
μ^+
γ
e^-
μ^-
a
$e^+ + e^- \rightarrow \mu^+ + \mu^-$
e^+
e^+
γ
+
e^-
e^-
b
$e^+ + e^- \rightarrow e^+ + e^-$
c
e^+
γ
q
$\bar{q}$
e^-
HADRONS
$e^+ + e^- \rightarrow$ hadrons

Fig. 2 Diagrammatic representation of the one photon contribution to the reactions: *a*, $e^+ + e^- \rightarrow \mu^+ + \mu^-$; *b*, $e^+ + e^- \rightarrow e^+ + e^-$ and *c*, $e^+ + e^- \rightarrow$ hadrons through production of a quark–antiquark pair.

Table 3 lists the assumed properties of the four quarks and four antiquarks. The existence of a fourth quark is also required to explain the absence of neutral current effects in some regions of the weak interaction (in neutral K^0 decays) The new particles are still under investigation and theoretical problems remain but there is a strong feeling that high energy physics is on the point of a breakthrough.

Why EPIC?

EPIC[10] is an accelerator in which high energy (14 GeV) positrons are collided head-on with electrons of the same energy. The whole energy of the electron and positron is available in the centre-of-mass system. In contrast if a positron hits a stationary electron essentially all of the energy is used in producing motion of the centre of mass and to achieve 28 GeV centre-of-mass energy in this way would require a positron energy of 800,000 GeV.

Why is it necessary or desirable to study e^+e^- reactions? Are not pp studies adequate? e^+e^- reactions are predominately electromagnetic. The annihilation of an initial e^+e^- state to give any final state is dominated by the exchange of a single virtual photon as indicated in Fig. 2*a* for the reaction $e^+ + e^+ \rightarrow \mu^+ + \mu^-$, in Fig. 2*b* for the two contributions to $e^+ + e^- \rightarrow e^+ + e^-$, and in Fig. 2*c* for the reaction $e^+ + e^- \rightarrow$ hadrons on the assumption that production of hadrons is a result of the production of a quark–antiquark pair. In all cases the contribution from the exchange of two photons is of the order of 1%. The domination of the single photon contribution means that the final state has a well defined quantum state with a given spin, parity and charge quantum number ($J^{PC} = 1^{--}$). In contrast the pp reaction with a centre-of-mass energy of 28 GeV would have contributions from a hundred different values of *J*. The uniqueness of the final state brings strong constraints and rather precise predictions on what is expected. Experiments can therefore check the basic assumption that QED works to distances of $\sim 10^{-16}$ cm: that is, to volumes of 10^{-9} of the proton volume. The energy density reached in these reactions is $\gtrsim 10^{19}$ GeV cm^{-3}—orders of magnitude larger than that found in astrophysics—and we have no right to assume that laws tested elsewhere will of necessity still apply in this new energy regime.

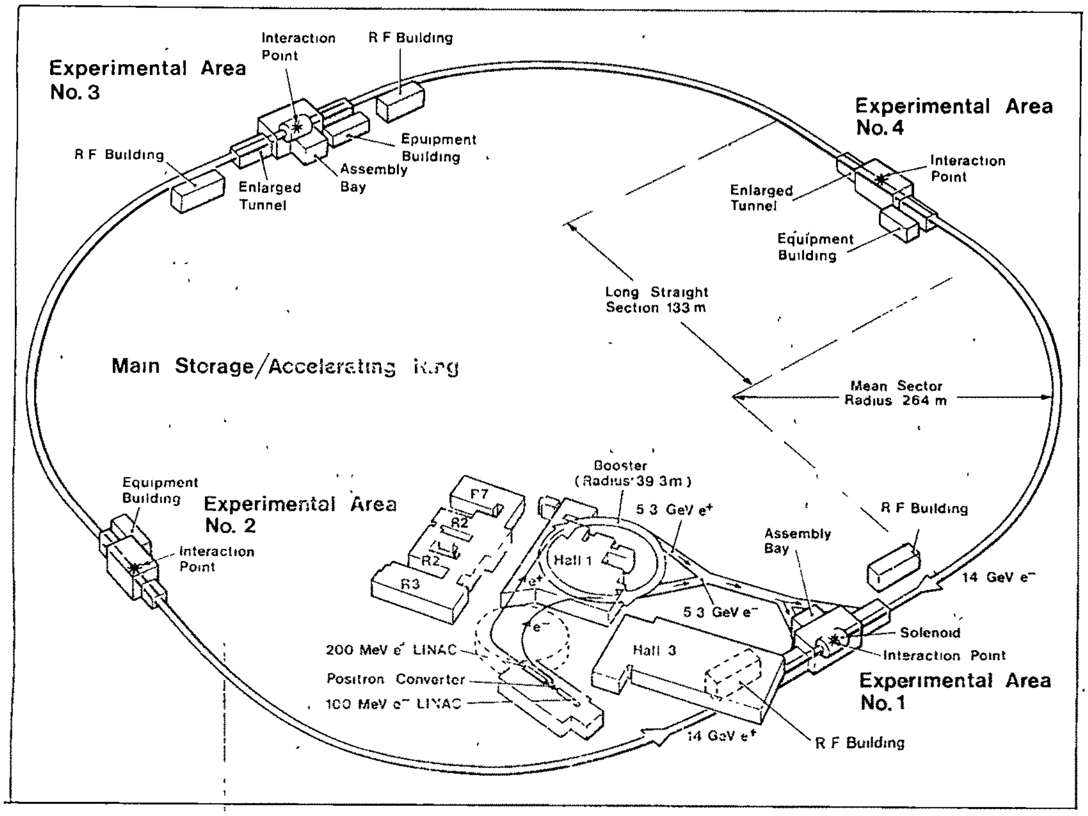


Fig. 3 Diagram of EPIC showing major components. The booster, one of the LINACS and the buildings shown in the booster area already exist.

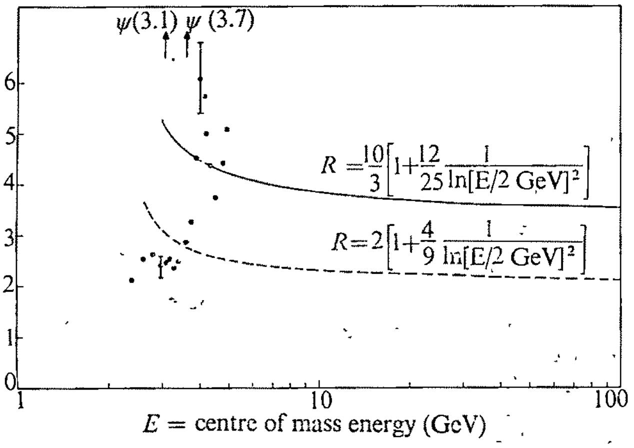


Fig. 4 Experimental values of

$$R = \frac{\text{cross section for } e^+ + e^- \to \text{hadrons}}{\text{cross section for } e^+ + e^- \to \mu^+ + \mu^-}$$

and the theoretical predictions for two different quark models The lower energy region includes resonances. At EPIC energies one should be close to the asymptotic values and should be able to 'determine' the form of the substructure of hadrons.

At EPIC energies one can expect to see contributions from other interactions—from the weak interaction caused by the exchange of neutral vector bosons—from the strong interaction testing the properties of the constituent parts and probing their structure to distances $\sim 10^{-16}$ cm. The existence of a good theory for the electromagnetic reaction provides a fine probe for investigating the unknown: it is analogous to probing the structure of a watch using X rays rather than by throwing two watches at each other and studying the resulting parts, which is closer to the situation when one studies pp reactions.

Achieving electron–positron collisions

If a beam of electrons is fired at a beam of positrons the majority of the particles miss each other because the cross sections are very small ($\sim 10^{-34}$ cm^2) at EPIC energies. To compensate for this the particles are contained within a toroidal vacuum chamber by means of guiding magnetic fields so that they can be reused. The electrons rotate one way and the positrons counter-rotate within the same vacuum chamber. Bending the particles around to reuse them results in energy loss by synchrotron radiation (emission of short wavelength light) and on average each electron and positron loses 20 MeV per revolution. 3×10^{12} particles are stored in EPIC and they make 1.4×10^5 circuits per s resulting in $\sim 10^{19}$ MeV lost per s or 1.4 MW. This loss has to be compensated by accelerating cavities operating at about 400 MHz—about 3–4 MW of power are provided to the cavities to replace the 1.4 MW lost by the beams. The energy lost per turn by an electron is $\propto E^4/R$ where E is the energy of the electron and R is the radius of the machine. It is this fact that limits the energy that can be used and forces the radius of EPIC to such a large value (Fig. 3). The circumference of EPIC is 2.2 km.

The radio frequency acceleration bunches the electrons and positrons. In EPIC there are two bunches of electrons diametrically spaced and two similarly spaced bunches of positrons rotating in the opposite direction. The particles are phased so that they cross in the centre of the four long straight sections (Fig. 3). At these four interaction points the bunches of particles are strongly focused to increase the interaction rate—the vertical height at this point is ~ 0.015 cm, the horizontal width is 0.06 cm and the length ~ 10 cm. It is not possible to focus the bunches even more strongly because of beam–beam interactions which are dependent on current density. The interaction rate is determined by the properties of storage ring and the cross section to be studied, so that

$$\text{Interaction rate} = \sigma L$$

L is the luminosity of the storage ring and the design value for EPIC at 28 GeV centre-of-mass energy is 5×10^{31} cm^{-2} s^{-1}. For a typical cross section of 10^{-34} cm^2 this gives a count rate of 18 h^{-1} at each intersection region. This count rate is not large but is quite adequate to do the physics envisaged. Some cross sections are much larger and have higher event rates.

Electrons and positrons have to be injected into the EPIC main ring and the plan is to use the 5 GeV electron accelerator NINA and associated equipment. NINA will be moved from the Daresbury Laboratory to the Rutherford Laboratory when the physics programme at Daresbury ends in 1977. Buildings and ancillary plant currently used for the 7 GeV proton accelerator NIMROD at the Rutherford Laboratory will also be used wherever possible. It is planned to stop operation of NIMROD in 1979 and EPIC should be ready to start commissioning its first physics experiments in 1980.

The accumulation of the required 8×10^{11} positrons in each of the two bunches in EPIC represents the principal problem in the injection process. Electrons are accelerated to 0.1 GeV in a linear accelerator and are focused on to a metal target resulting in the production of electron–positron pairs. The positrons are collected and accelerated to 0.2 GeV in a second linear accelerator. These positrons are further accelerated in NINA to an energy of 5.3 GeV before they are injected into EPIC. About ten thousand such cycles are needed to accumulate the required number of positrons and this takes about three minutes. The filling of the electron bunches in EPIC is easier as the positron conversion efficiency is about 10^{-3} and thus for electrons very few NINA acceleration cycles are required. The total filling time is expected to be less than four minutes

The two bunches of electrons and two bunches of positrons in EPIC are accelerated from 5.3 GeV to 14 GeV in about one minute and the full energy beams can then be stored for several hours while interactions take place at the four interaction regions. The beams are gradually attenuated as a result of the beam–beam interactions and the scattering on the residual gas even though the vacuum is kept at 10^{-9} mmHg.

If more radio frequency cavities are added it is possible to increase the energy of EPIC to about 20 GeV per beam, 40 GeV centre-of-mass energy, but at a reduced luminosity.

What experiments can one do with EPIC?

The potential contribution of EPIC in high energy physics is fourfold. First, it may help to answer some outstanding questions on hadron substructure. Experimental results on e^+e^- annihilation into hadrons made at the SPEAR storage ring at the Stanford Linear Accelerator Laboratory, USA, and at other lower energy storage rings show that the cross section for the production of hadrons is much larger than would originally have been expected[2,8]. The large cross sections are direct evidence that the hadron production occurs through some particles of very small dimension; the current assumption is that it is through production of a quark–antiquark pair (see Fig. 2*c*). This model predicts that the ratio

$$R = \frac{\text{cross section for } e^+e^- \to \text{hadrons}}{\text{cross section for } e^+e^- \to \mu^+\mu^-} = \sum_i q_i^{\,2}$$

where q_i is the electronic charge of the quark and the summation is overall possible quarks. Thus for the three quark model one predicts $R = (\frac{1}{3})^2+(\frac{1}{3})^2+(\frac{2}{3})^2 = \frac{2}{3}$ and for the four quark model $R = (\frac{1}{3})^2+(\frac{1}{2})^2+(\frac{2}{3})^2+(\frac{2}{3})^2 = 10/9$.

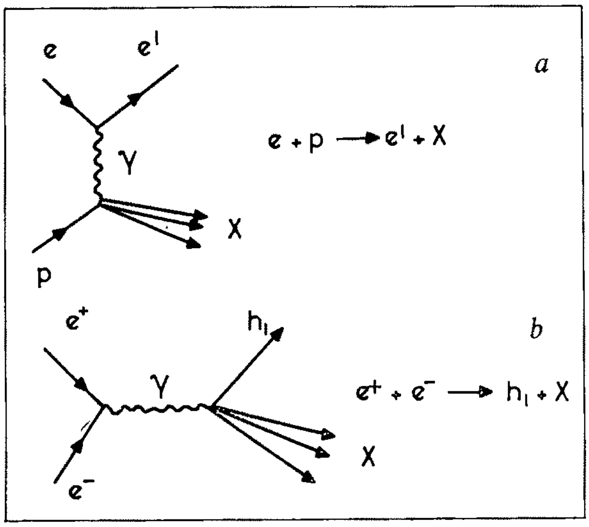


Fig. 5 *a*, Reaction $e+p \rightarrow e'+$anything. It can be thought of as a massive virtual photon probing the structure of the proton. As the probed particle is the target only neutrons and protons can be studied this way. *b*, Represents the reaction $e^{+}+e^{-} \rightarrow h_{l}+$ anything. Similarly this may be thought of as a massive virtual photon probing the structure of the hadron h_{l}. Any hadron that is produced can be studied in this way including unstable particles such as pions, kaons.

There are more complicated quark models predicting that three varieties of each quark should exist[11] with an additional label: colour. For such a model without charm $R = 2$, with both charm and colour $R = 10/3$. More complicated models[12] exist that predict even larger values of R

The prediction is that at high energies R should be independent of energy and that its value is dependent on the assumed building blocks for the hadrons. At lower energies resonances will be seen corresponding to the production of particular hadron states: the new particles at 3.1, 3.7 and 4.2 GeV are examples of this. There are model dependent predictions on the approach to the asymptotic value of R and Fig. 4 shows these predictions[13] and the existing experimental results. EPIC will be able to reach the asymptotic region and will determine which, if any, of the quark models is correct.

More information can be obtained by studying the details of the particle production. As mentioned above, deep inelastic electron scattering, $e+p \rightarrow e'+$anything, has been used to probe the structure of the proton and Fig. 5*a* shows the reaction diagrammatically: the virtual photon probes the structure of the proton. Figure 5*b* shows the reaction $e^{+}+e^{-} \rightarrow h_{l}$ + anything where h_{l} can be any hadron (for example a proton, a pion, a kaon and so on). The reactions are related and one can think of reaction 5*b* as probing the structure of the hadron h_{l}. This process has the advantage of being able to probe unstable as well as stable hadrons whereas inelastic electron scattering requires a stable target and is thus limited to protons or neutrons. Measurements will include single particle production to test scaling; determination of particle ratios as a function of particle momentum; determination of particle correlations; measurement of specific exclusive channels and so on. EPIC opens a new range of energies for such studies and allows a much deeper investigation of hadron substructure than is currently possible.

The second issue which EPIC might help to resolve is the distance over which quantum electrodynamic equations are valid. The experimental[2] values of $\sigma(e^{+} + e^{-} \rightarrow \text{hadrons})$ is constant as $\sim 2 \times 10^{-32}$ cm² for centre-of-mass energies from 3 to 5 GeV. Should this value continue to 28 GeV strong interaction corrections to the photon propagator would produce measurable effects and a breakdown of the normal QED perturbation expansion would be evident[14].

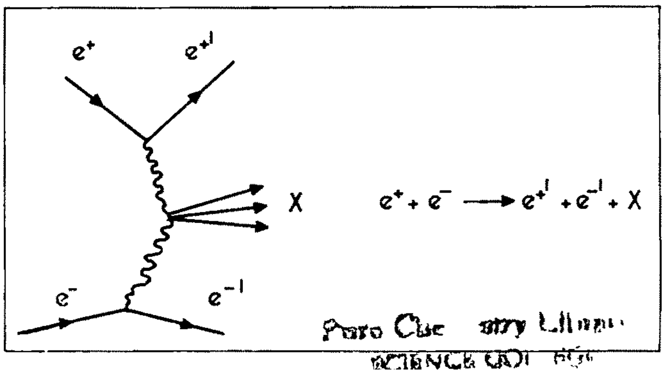
There are three main reactions that can be used to test QED at EPIC; $e^{+} + e^{-} \rightarrow e^{+} + e^{-}$; $e^{+} + e^{-} \rightarrow \mu^{+} + \mu^{-}$ and $e^{+} + e^{-} \rightarrow 2\gamma$. All can be studied experimentally and QED makes detailed predictions about the cross sections and angular distributions expected. Such measurements will test the validity of QED to distances $\sim 10^{-16}$ cm—a factor of ten further than currently possible. Different experimental conditions can be chosen to check photon and lepton propagators over a wide range of momentum transfers.

Third, EPIC energies could clarify outstanding problems with the weak force. The reaction $e^{+} + e^{-} \rightarrow \mu^{+} + \mu^{-}$ is dominated by the exchange of a single photon (Fig. 2*a*). Contributions are also possible from the weak interaction through the exchange of a neutral intermediate boson. At EPIC energies this effect is about 3% but there is a further contribution from interference between the weak interaction and the electromagnetic interaction that is $\sim \sqrt{3}\% \simeq 17\%$. The exact level of the effect is dependent on the form of the neutral current contribution to the weak interaction. The ratio of the weak interaction contribution to $e^{+} + e^{-} \rightarrow \mu^{+} + \mu^{-}$ to that from the electromagnetic interaction increases as the fourth power of the centre-of-mass energy and thus it is important to run EPIC at as high an energy as possible. The weak interaction modifies the QED predictions by changing the rate for the reaction, changing the angular distribution and by producing polarisation effects.

Such effects can be measured even if the mass of the neutral mediator of the weak interaction is too large for the particle to be directly produced. Different unified theories for the weak and electromagnetic interactions make specific predictions. For example the Weinberg–Salam model predicts a particle Z_0 of mass greater than 79 GeV, but its existence produces detectable effects in the $e^{+} + e^{-} \rightarrow \mu^{+} + \mu^{-}$ reaction at EPIC energies. Experiments will thus be able to test these predictions, decide which model is correct and possibly determine the mass of the neutral mediator.

So far I have concentrated on the domination of one photon exchange in annihilation of $e^{+}e^{-}$. The fourth area in which EPIC might make an important contribution is that of non-annihilation reactions in which two-photon contributions dominate. Such reactions are $e^{+} + e^{-} \rightarrow e^{+} + e^{-} + X$ where X stands for any final state. Figure 6 represents this diagrammatically. The reaction can be thought of as the collision of two virtual photons to form the final state X. The cross section for such reactions can be large and the reactions can be identified by detecting and measuring the e^{+} and e^{-} in the final state. A whole class of interesting physics can be studied in this way that cannot be tackled by other means. At available storage rings the cross sections are small but at EPIC energies

Fig. 6 Representation of the reaction $e^{+}+e^{-} \rightarrow e^{+\prime}+e^{-\prime}+X$, where X represents any final state. Different energies and directions of the final state inelastically scattered electron and positron ($e^{+\prime}$ and $e^{-\prime}$) and final states X can be selected to study a wide range of important physics.



they are predicted to exceed the annihilation channels by more than a factor of 100.

One of the most interesting studies is to choose the energies and direction of the e^+ and e^- in the final state so that one of the virtual photons is almost real (a mass less than the mass of the pion) and the second virtual photon is massive. This corresponds to a virtual photon probing the structure of the photon itself. Does the photon have constituent parts?

Feasibility of EPIC

The UK high energy physics community has put a great deal of effort into a feasibility study of EPIC[10,15]. The cost of the project is estimated to be £25.7 million. In spite of projected decreases in the funds for Nuclear Physics, the Nuclear Physics Board of the Science Research Council has fitted the project within its future plans by planning to close the existing two domestic high energy physics accelerators NINA and NIMROD in 1977 and 1979 respectively. The operation and utilisation costs for these accelerators is diverted into construction money for EPIC and ultimately into operation and utilisation money for the single facility.

The Science Research Council has accepted that there is a strong scientific case for the construction of EPIC and has included it within its future plans provided that some international contribution can be found towards its construction and operation.

Within Europe there is a second proposal for a high energy e^+e^- colliding beam machine—namely PETRA[16], which is proposed by the DESY Laboratory in Germany. European high energy physicists as represented by ECFA (European Committee for Future Accelerators) have recommended that one or other of the two projects should be supported and constructed. It is generally accepted that whichever is built international participation in the utilisation is certain.

British physicists argue that EPIC is to be preferred as DESY should concentrate on developing and using their lower energy storage ring DORIS that is just starting operation. A further argument in favour of EPIC is that it has been designed so that a magnet ring for protons can be added above the electron–positron ring and within the same tunnel. This addition will allow for the study of ep reactions. This is an option for the future that is not available in the PETRA design.

British high energy physicists argue strongly that an opportunity exists for a unique and exciting project to be constructed in the UK. The physics that can be studied is at the forefront of our understanding. It is in an area that we have the necessary expertise within the universities and the Science Research Council to exploit. German physicists argue equally strongly for the constructiom of PETRA in Germany. The choice is difficult, but it is important that a decision should be made and that a European e^+e^- project should be started as soon as possible.

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articles

Methyl group as a probe of chirality in Raman optical activity

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Raman circular intensity differentials observed in methyl asymmetric deformations and methyl torsions indicate that the methyl group could function as a powerful new probe of chirality. Torsional Raman optical activity could lead to new knowledge of barriers to internal rotation in molecules and aid the assignment of torsional modes.

VIBRATIONAL optical activity can be studied through a difference in the intensity of Raman scattering in right and left circularly polarised incident light (see refs 1 to 5). The effect originates in interference between light waves scattered through the electronic polarisability and the electronic optical activity of the molecule. An appropriate experimental quantity is a normalised circular intensity differential (CID),

$$\Delta_\alpha = (I_\alpha^R - I_\alpha^L)/(I_\alpha^R + I_\alpha^L)$$

where I_α^R and I_α^L are the scattered intensities with α polarisation in right and left circularly polarised incident light. (I_z and I_x are linearly polarised parallel and perpendicular to the scattering plane and Δ_z and Δ_x are referred to as the depolarised and polarised CIDs.) Routine Raman CID spectrometers have been described elsewhere (see refs 4 and 5).

Raman CIDs have been observed in many molecules, but because of the complexity of the spectra and the considerable mixing of the normal vibrational coordinates in the regions where the best effects are usually found (0–1,500 cm^{-1}), it has been difficult to assign the Raman CIDS to particular normal

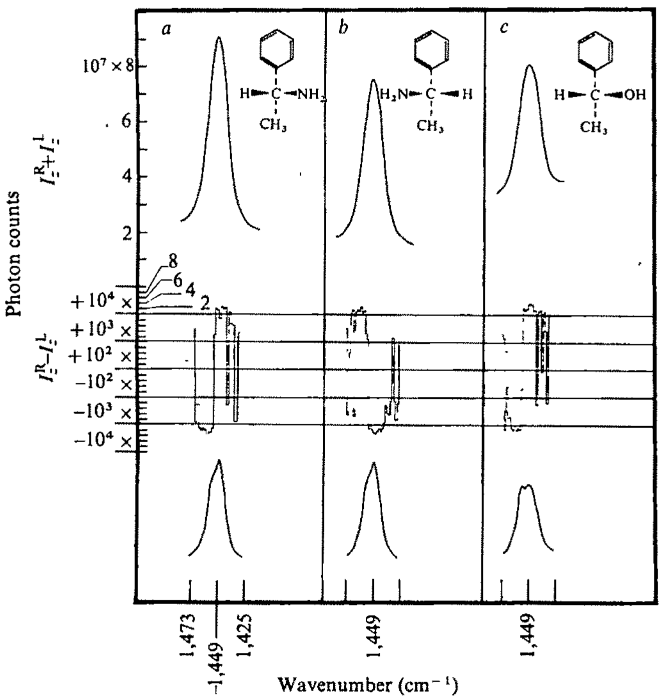

Fig. 1 The depolarised Raman circular intensity sum (top) and difference (centre) spectra, as recorded, of methyl asymmetric deformations in: *a*, (+)-α-phenylethylamine, *b*, (−)-α-phenylethylamine; *c*, (+)-α-phenylethanol. At the bottom is a sum spectrum at high resolution. Instrumental conditions: laser wavelength 4,880 Å, laser power 1 W, slit width 1,500 μm for the top and centre spectra and 200 μm for the bottom spectra. The absolute configurations are taken from ref. 10.

coordinates of vibration. In general, cyclic structures show larger effects than acyclic structures in which the existence of rotational conformers can reduce the chirality, although exceptions include molecules where four rigid groups are attached to an asymmetric carbon atom as in α-phenylethylamine.

Definite assignments of Raman optical activity to localised vibrations have now been made in the important case of the methyl group, and examples of Raman CIDs generated by methyl asymmetric deformations and methyl torsions are shown below. (For reasons discussed previously[3,5], only the depolarised CIDs are sampled. To estimate the normalised CIDs, the backgrounds should first be subtracted from the $I_z^R+I_z^L$ spectra.) By means of these effects, the methyl group could function as a specific and reliable probe of configuration, although there are insufficient data at present to attempt detailed correlations. Similar assignments will probably be made for vibrations in other groups, in particular deformations and torsions of $-C_6H_5$, $-NH_2$, $-NH_3^+$, $-OH$, $-NO_2$, and so on.

Methyl asymmetric deformations

Raman CID couplets are found in a depolarised band at about 1,450 cm^{-1} in α-phenylethylamine and α-phenylethanol and the spectra, as recorded, are shown in Fig. 1.

This effect in α-phenylethylamine was first noticed by Hug *et al.*[4], who realised that it originated in the two degenerate asymmetric deformations of the methyl group and could function as a new probe of chirality. If the effective symmetry of the methyl group is reduced through bonding such that its threefold axis is lost, the degeneracy is lifted and a splitting can sometimes be seen in the infrared or Raman spectrum[6]. This splitting is visible in the Raman spectra of α-phenylethylamine and α-phenylethanol at high resolution (200 μm), but not at the low resolution (1,500 μm) used to scan the CIDs (Fig. 1).

The effective symmetry of a methyl group bonded to an asymmetric carbon atom is C_1, so its electronic and vibrational transitions can support optical activity; here this takes the form of Raman CIDs of opposite signs in the two components of the methyl asymmetric deformation.

Similar effects should exist in molecules such as alanine, lactic acid and threonine in aqueous solution, but are below the present limits of detection; also in $-NH_3^+$ attached to asymmetric carbon atoms in all amino acids in acid aqueous solution. Examples of this effect are not as distinctive in methyl groups in molecules such as 2-amino-l-phenylpropane (amphetamine) and 2-octanol because of the existence of rotational conformers, nor in methyl groups attached to ring structures.

Methyl torsions

Raman CIDs are found in methyl torsion vibrations between about 200 and 300 cm^{-1} in most optically active molecules containing methyl groups. For example, (+)-3-methylcyclohexanone shows a broad weak depolarised Raman band at about 260 cm^{-1} with a large CID ($\Delta_z \sim -2.5\times10^{-3}$, Fig. 2*a*); this can be assigned to the methyl torsion which is known to occur between about 230–280 cm^{-1} in methylcyclohexanes[7]. The corresponding band in (+)-3-methyl-cyclopentanone may be that at about 290 cm^{-1} ($\Delta_z \sim -1.5\times10^{-3}$, Fig. 2*b*). Similar bands appear in (−) limonene ($\Delta_z \sim -1.5\times10^{-3}$, Fig. 2*c*) and (+) carvone ($\Delta_z \sim +1.8\times10^{-3}$, Fig. 2*d*), but now the interpretation is no longer unequivocal since both molecules possess two methyl groups. It is possible that the bands shown at about 250 cm^{-1} originate in the isopropenyl methyl group since the corresponding torsional barrier, and hence the torsional frequency, is expected to be higher than for the ring methyl group (methyl torsions in *ortho*-substituted toluenes occur around 170 cm^{-1}, ref. 8).

A dramatic example of torsional Raman optical activity is found in dimethyldibenz-1,3-cycloheptadiene-6-one (Fig. 2*e*), a bridged biphenyl in which two equivalent methyl groups twist in a highly chiral environment. Coupling between the two methyl torsions generates two Raman bands, associated with symmetric and asymmetric combinations of the local modes, centred at about 234 cm^{-1}. An enormous Raman CID ($\Delta_z \sim -7\times10^{-3}$) is found in the lower frequency band, but none in the higher frequency band.

Unfortunately torsional modes other than methyl are often difficult to assign unambiguously as the frequencies are very variable and can overlap with low frequency skeletal modes. On the other hand, Raman optical activity could be of considerable assistance in assigning torsional modes in optically active molecules. In fact the first reported observations[3] of Raman

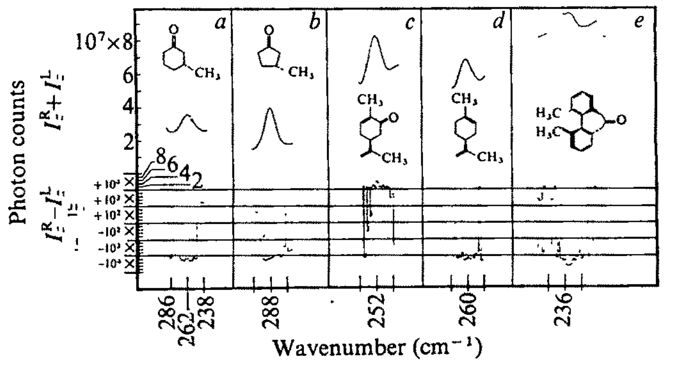

Fig. 2 The depolarised Raman circular intensity sum and difference spectra of methyl torsions in; *a*, (+)-3-methylcyclohexanone; *b*, (+)-3-methylcyclopentanone; *c*, (+)-carvone; *d*, (−)-limonene; *e*, a saturated solution of (+)-dimethyldibenz-1, 3-cycloheptadiene-6-one in acetone. Instrumental conditions as for the top two spectra in Fig. 1, except that a laser wavelength of 5,145 Å was used in *e* to minimise fluorescence.

CID in α-phenylethylamine and α-phenylethanol between about 200 and 400 cm^{-1} probably involve $-CH_3$, $-NH_2$ and $-OH$ torsions.

Raman optical activity is determined by products such as[1,5]

$$\langle \nu^1 | \alpha_{\alpha\beta} | \nu^f \rangle \langle \nu^f | G'_{\alpha\beta} | \nu^1 \rangle$$

$$= \sum_J \left(\frac{\partial \alpha_{\alpha\beta}}{\partial Q_J} \right)^*_e \left(\frac{\partial G'_{\alpha\beta}}{\partial Q_J} \right)_e | \langle \nu^f | Q_J | \nu^1 \rangle |^2$$

where ν^1 and ν^f are the initial and final vibrational states, Q_J is the Jth normal vibrational coordinate, $\alpha_{\alpha\beta}$ is the electric dipole–electric dipole polarisability tensor, $G'_{\alpha\beta}$ is the electric dipole–magnetic dipole optical activity tensor and $A_{\alpha\beta\gamma}$ is the electric dipole–electric quadrupole optical activity tensor. So the Raman CID associated with the methyl torsion originates in the products of the changes in electronic polarisability and electronic optical activity, evaluated at the equilibrium nuclear configuration, as the methyl group twists about its threefold axis in the chiral environment from the rest of the molecule. For 'static coupling' of the methyl group with the chiral environment, a sextant symmetry rule is predicted[2].

Since torsional Raman optical activity arises from the chiral part of the electronic potential barrier hindering the motion, it could provide a novel approach to the study of internal rotation in molecules, and to conformational analysis generally. For example, in the chair form of 3-methylcyclohexanone with the methyl group occupying the equatorial position, most of the potential barrier hindering the methyl torsion arises from the two flanking methylene groups. The presence of the sp^2 hybridised atom in the ring results in an unsymmetrical chair so that the two methylenes flanking the methyl are slightly twisted relative to each other. In the (+) enantiomer the twist is left-handed and is probably responsible for the negative methyl torsional Raman optical activity, although the theory is not sufficiently developed to predict with confidence the sign from the sense of twist. It is therefore possible that the negative methyl torsional optical activity in (+)-3-methylcyclopentanone arises because in this molecule too there is a left-handed twist in the two methylenes flanking the methyl. Although little is known about the conformations of five-membered rings, this could be consistent with the suggested requirements[9] for the methyl to occupy the quasi-equatorial position in the envelope form of methylcyclopentane and for ring puckering at the carbon atoms away from the sp^2 hybridised atom in cyclopentanone.

I thank the Ramsay Memorial Fellowships Trust for support, Dr D. G. Buckley for preparing (+)-dimethyldibenz-1, 3-cycloheptadiene-6-one from a sample of (—)-6,6′-dimethyl-2, 2′-diphenic acid supplied by Professor K. Mislow, and Professor A. D. Buckingham, Dr W. J. Jones and Professor W. Klyne for comments.

Received March 19; accepted April 28, 1975.

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Stringent control of the transcriptional activities of ribosomal protein genes in *E. coli*

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The stringent control system regulates the expression of not only rRNA genes but also r-protein genes. The regulation of r-protein genes takes place by a mechanism which determines the amount of r-protein mRNA available for translation. Presumably this mechanism operates at the level of initiation of transcription at r-protein gene promoters.

IN the bacterium *Escherichia coli*, the synthesis of ribosomal components is regulated[1,2]. One aspect of this regulation which has been studied extensively is the stringent control of stable RNA synthesis; during amino acid (or charged tRNA) deprivation in stringent (rel^+) strains stable RNA accumulation is strongly depressed, whereas in mutant relaxed (rel^-) strains RNA accumulation continues unabated[2-4]. In contrast to stable RNA, it has been thought that amino acid starvation does not affect the synthesis of mRNA to any great extent[3-6].

We have shown previously that the synthesis of ribosomal proteins (r-proteins) like rRNA is subject to the influence of the stringent control system[7]. We used isogenic rel^+ and rel^- strains carrying a temperature sensitive mutation in the valyl-tRNA synthetase gene (*ValS*); at growth temperatures above 32 °C the level of amino acylated valyl-tRNA becomes deficient and therefore the effect of charged tRNA deprivation on RNA and protein synthesis can be studied. It was observed that during partial inhibition of tRNA charging in these strains, the synthesis of individual r-proteins remained in balance and closely coupled to the accumulation of stable RNA[7]. That is, in the rel^+ strain, the synthesis of stable RNA (that is, accumulation) and r-protein was more strongly inhibited than total protein synthesis and the differential rate of r-protein synthesis, α_r (r-protein synthesis rate/total protein synthesis rate), was reduced; in the rel^- strain the synthesis of stable RNA and r-protein was stimulated relative to the synthesis of total protein and α_r was increased. At more restrictive temperatures the coordination between the production of rRNA and r-protein in the rel^- strain begins to deteriorate because of the severe inhibition of protein synthesis, although α_r continues to increase. Presumably relaxed particles are accumulated in these restrictive conditions[8].

To elucidate further the nature of the coordinate expression of rRNA and r-protein genes in rel^+ and rel^- bacteria we have determined directly the amount of mRNA coding for r-protein during various degrees of amino acid deprivation. Partial or complete amino acid deprivation was accomplished by shifting

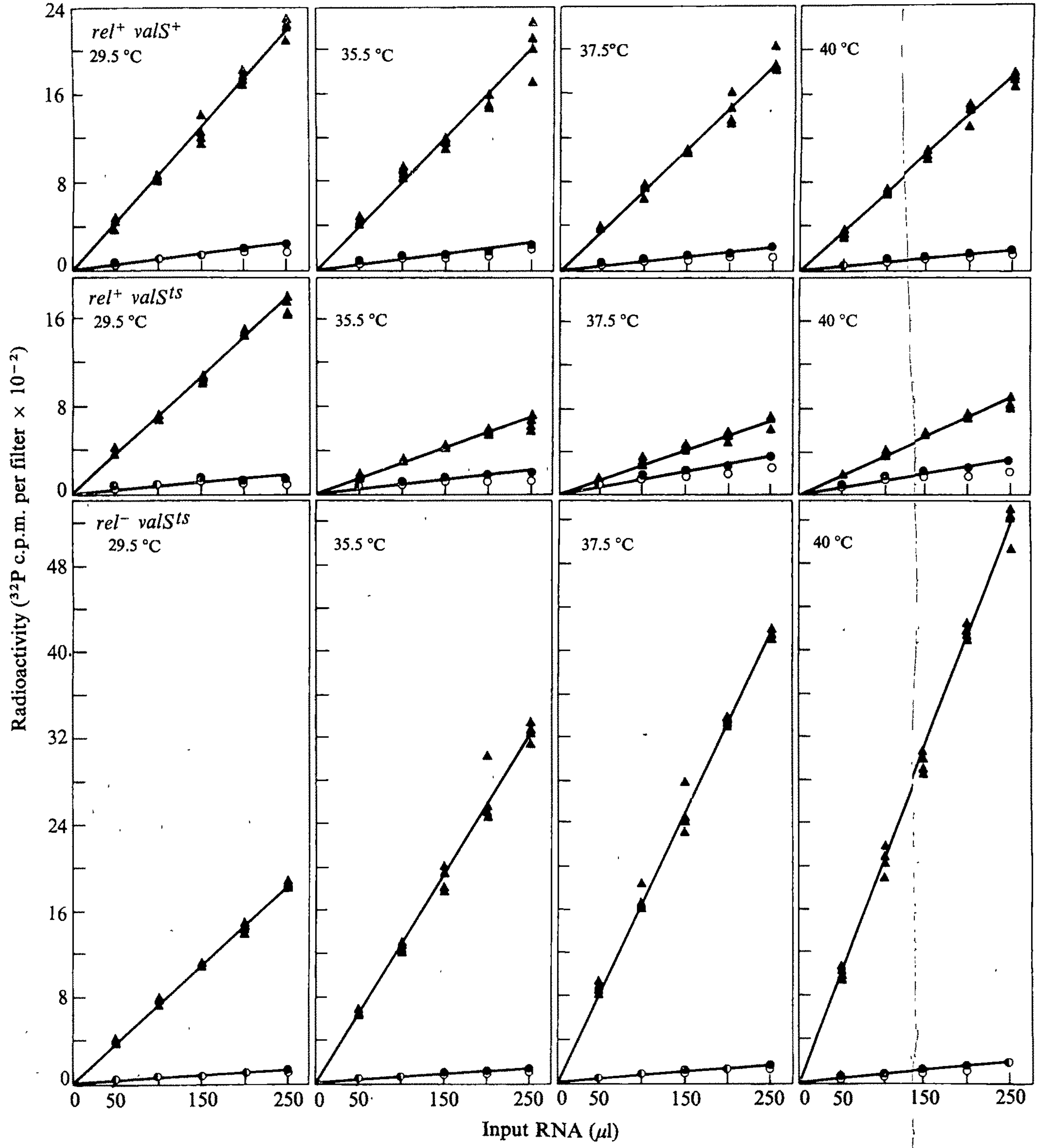

Fig. 1 Hybridisation of total cellular RNA to DNA from λ*dtrk* and λ*dspc*1 transducing phages. The bacterial strains which require leucine were grown in minimal morpholinopropane sulphonic acid (MOPS)[10] medium supplemented with 0.2% glucose, leucine (20 μg ml^{-1}), and 0.2 mM phosphate (specific activity 50 mCi mmol^{-1}) at 29.5 °C in a rotary shaker bath for 20 cell doublings to label homogeneously the cellular nucleic acid. The doubling times of the bacterial mass in the cultures were monitored as absorbance at 460 nm and were between 65 and 70 min. At an A_{460} of 0.2–0.3, portions of the culture were shifted to rotary water baths set at 35.5, 37.5 or 40 °C. After 15 min the bacteria were collected and total cellular RNA was prepared. DNA was extracted from λ phages and transducing phages λ*dtrk* and λ*dspc*1, denatured and immobilised on nitrocellulose membrane. The specific radioactivity of the RNA preparations was 144×10^7 c.p.m. per A_{260} and the concentration in A_{260} U ml^{-1} at the respective incubation temperatures of 29.5, 35.5, 37.5 and 40 °C were: $valS^+rel^+$ (NF314) 1.06, 1.08, 1.08 and 1.12; $valS^{ts}rel^+$ (NF536) 1.11, 1.08, 1.10, 1.09; $valS^{ts}rel^-$ (NF537) 1.07, 1.08, 1.08 and 1.05. RNA (50–250 μl) was hybridised in a total volume of 2 ml of 2×SSC to two nitrocellulose filters containing no DNA, two filters containing 5 μg each of λDNA, two filters containing 5 μg each of λ*dtrk* DNA and four filters containing 5 μg each of λ*dspc*1 DNA for 18 h at 67 °C. After hybridisation, the filters were washed with 2×SSC, treated with T_1 RNase and RNase A, and counted in 5 ml of toluene as scintillation fluid. Radioactivity associated with the blank filters ranged from about 50 to 250 counts per filter with increasing input RNA and were subtracted from the radioactivity associated with the λDNA filters (○), λ*dtrk* DNA filters (●) and λ*dspc*1 DNA (▲) filters. The amount of specific hybridisation is obtained as four times the difference between the λ*dspc*1 and λ*dtrk* curves. The factor four accounts for the four separate filters each containing 5 μg of λ*dspc*1 DNA in each hybridisation assay.

Table 1 Fraction of the total cellular RNA which is specific mRNA for r-protein coded for by genes on the λ*dspc*1 phage

Bacterial strain	Incubation temp. (°C)	Specific hybrids (c.p.m. × 10^{-3})	Input RNA (c.p.m. × 10^{-6})	$\frac{\text{Specific hybrids}}{\text{Input RNA}}$ (× 10^{3})	Specific r-protein mRNA fraction (%)
$valS^{+}rel^{+}$ (NF312)	29.5	7.85	3.82	2.06	0.275
	35.5	7.14	3.89	1.83	0.244
	37.5	6.85	3.89	1.76	0.234
	40.0	6.49	4.03	1.61	0.215
$valS^{ts}\,rel^{+}$ (NF536)	29.5	6.66	4.00	1.67	0.223
	35.5	1.96	3.89	0.50	0.067
	37.5	0.91	3.96	0.23	0.030
	40.0	2.10	3.93	0.53	0.070
$valS^{ts}\,rel^{-}$ (NF537)	29.5	6.29	3.85	1.63	0.217
	35.5	12.40	3.89	3.19	0.425
	37.5	15.95	3.89	4.11	0.548
	40.0	19.90	3.78	5.27	0.704

The radioactivity in specific hybrids was obtained from the experiments illustrated in Fig. 1 and is equal to four times the difference between radioactivity hybridised to λ*dspc*1 DNA and λ*dtrk* DNA. The factor four accounts for the four separate λ*dspc*1 DNA filters each containing 5 μg of DNA. Values calculated for a total of 20 μg λ*dspc*1 DNA are given in the table (see below). The calculation was made at an input of 250 μl of RNA and the radioactivity in the input RNA was calculated from the information in the legend of Fig. 1. The fraction of the total RNA which is mRNA coded for by the r-protein genes carried in the λ*dspc*1 transducing phage was obtained using the relationship:

$$sK = H/R$$

where s is the fraction of specific r-protein mRNA; H is radioactivity in specific λ*dspc*1 hybrids; R is radioactivity in input total RNA; K is a constant $\simeq 0.75$ The value of the constant K depends on the conditions of hybridisation and accounts for the fraction of the specific RNA which enters into hybrids with λ*dspc*1 at equilibrium. In our condition of hybridisation (20 μg of λ*dspc*1 DNA per assay) we have found that the numerical value of K is between 0.75 and 0.90. The calculation is based on the lower estimate of 0.75 and may thus contain a systematic overestimation of the fraction of specific r-protein mRNA by as much as 10–20% (our unpublished result).

isogenic rel^{+} and rel^{-} strains, carrying a temperature sensitive mutation in the *ValS* gene, from the permissive temperature of 29.5 °C to the semipermissive or restrictive temperatures of 35.5, 37.5 or 40 °C. The amount of mRNA coding for r-protein was determined by hybridisation of total cellular RNA, isolated from the bacterial strains after incubation at the various temperatures, with DNA from a lambda transducing phage carrying approximately half the r-protein genes from the bacterial chromosome[9]. These measurements clearly demonstrate that the stringent control system directly or indirectly regulates the amount of mRNA for r-protein as well as the rate of accumulation of stable RNA. This regulation may be exercised at the level of initiation of transcription at the promotors for the r-protein genes or alternatively the *rel* gene product may influence the stability of the mRNA for r-protein. Finally, we have also examined the nature of the residual protein synthesised at the restrictive temperature of 40 °C where inhibition of protein synthesis is virtually complete. The results indicate that r-protein represents a significant fraction of the residual protein synthesis in both the rel^{+} and rel^{-} strain and reflects the amount of r-protein mRNA detected by DNA–RNA hybridisation.

mRNA for r-proteins

Three isogenic strains were used: NF314, a non-temperature sensitive stringent strain ($leu^{-}valS^{+}rel^{+}$,parent); NF536, a temperature sensitive stringent strain ($leu^{-}valS^{ts}rel^{+}$) and NF537, a temperature sensitive relaxed strain ($leu^{-}\ valS^{ts}\ rel^{-}$). Cultures were grown at the permissive temperature of 29.5 °C in glucose minimal medium containing ^{32}P-orthophosphate. Portions of the cultures were shifted to 35.5, 37.5 and 40 °C. In the temperature sensitive strains the protein synthesis rate was initially reduced by about 10%, 80% and greater than 95% at the three respective temperatures. The classical stringent and relaxed responses of stable RNA accumulation were elicited in the rel^{+} and rel^{-} cultures at these elevated temperatures (refs 3 and 7 and Fig. 3).

The effect of the stringent control system on the expression of r-protein genes was examined by hybridising the total cellular RNA with DNA prepared from bacteriophage λ or transducing phages λ*dtrk* or λ*dspc*1. The λ*dtrk* DNA carries the *aroE* and *trkA* genes from the bacterial chromosome but no detectable r-protein genes, whereas the λ*dspc*1 phage carries the *aroE* and *trkA* genes in addition to approximately 22 r-protein genes from the cluster located near 64 min on the bacterial chromosome[9]. The r-protein genes on the λ*dspc*1 phage include S3, S4, S5 (*spc* gene product), S7, S8, S11, S13, S14, L5, L6, L11, L13, L14, L15, L16, L17, L18, L19, L22, L23, L24 and L30 and account for about 400,000 daltons of protein or about half of the aggregate protein per 70S ribosome.

Various amounts of the ^{32}P-RNA preparations were hybridised to a constant and excess amount of DNA from the bacteriophage λ, λ*dtrk* and λ*dspc*1 (Fig. 1). The amount of radioactivity entering into λ*dtrk* hybrids was only slightly greater than the nonspecific λ hybrids and suggests that the *aroE–trkA* region of the bacterial chromosome has a relatively low transcriptional activity. In contrast, at the permissive temperature the radioactivity entering into λ*dspc*1 hybrids was significantly greater than into the λ*dtrk* hybrids, indicating that the unique bacterial DNA carried by the λ*dspc*1 phage has a relatively high transcriptional activity. This transcriptionally active DNA represents r-protein genes and is virtually free of sequences which are homologous to stable RNA and other non-r-protein mRNAs (our unpublished results). Thus the difference between hybridisation to λ*dspc*1 and λ*dtrk* DNA has been termed specific r-protein mRNA and is homologous to the r-protein DNA region carried by the λ*dspc*1 phage.

The relative abundance of r-protein mRNA in the various RNA preparations is proportional to the slope of the specific hybridisation curves (Fig. 1 and Table 1). In the conditions of hybridisation used here, approximately 75–85% of the specific r-protein mRNA is in a DNA–RNA hybrid at equilibrium. Thus the absolute fraction of the total RNA preparation which is specific r-protein mRNA can be estimated (Table 1).

In the non-temperature sensitive parental strain the fraction of specific r-protein mRNA represents 0.21–0.24% of the total RNA and was essentially independent of the incubation temperature. In contrast, when the $valS^{ts}rel^{+}$ strain was shifted to 35.5, 37.5 or 40 °C, the fraction of specific r-protein mRNA decreased from a value of about 0.22% at 29.5 °C to an apparent minimum value of about 0.03% at 37 °C; when the $valS^{ts}rel^{-}$ strain was shifted to the respective temperatures, the fraction of specific r-protein mRNA gradually increased from a value of about 0.22% at 29.5 °C to a value of about 0.70% of the total RNA at 40 °C (Fig. 1 and Table 1). The

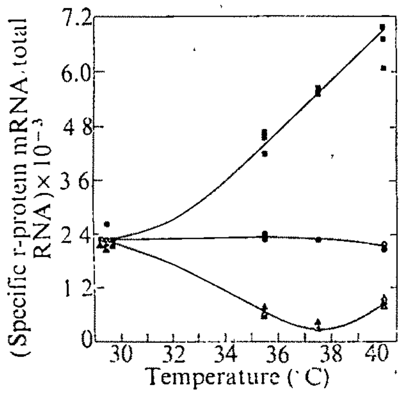

Fig. 2 Fraction of specific r-protein mRNA during partial or complete inhibition of valyl-tRNA aminoacylation. The *valS*⁺*rel*⁺ parental strain (●), the *valS*ᵗˢ*rel*⁺ strain (▲) and the *valS*ᵗˢ*rel*⁻ strain (■) were grown at the permissive temperature of 29.5 °C and shifted to semipermissive or restrictive temperature as described in Fig. 1. The fraction of the total RNA which is specific r-protein mRNA and homologous to the r-protein genes carried by the λ*dspc*1 phage was determined after incubation at 29.5 °C or 15 min after a shift to 35.5, 37.5 or 40 °C as described in Fig. 1 and Table 1.

results from several identical experiments of this type are summarised in Fig. 2.

During steady-state growth at the permissive temperature, r-protein accounts for about 13% of the total protein synthesis rate in these conditions[7] and the specific r-protein mRNA fraction is about 0.23% of the total cellular RNA. If all mRNA molecules are translated with equal efficiency and the r-protein genes carried by the λ*dspc*1 phage code for 50% of the total protein per ribosome, the amount of total mRNA can be calculated to be about 3.5% of the total cellular RNA.

After shifts from the permissive temperature of 29.5 °C to the semipermissive temperature of 35.5 °C, the accumulation of stable RNA and individual r-proteins remains closely coupled[7]. In the *valS*ᵗˢ*rel*⁺ strain the rates of stable RNA and r-protein accumulation decrease to about 60% of control values at 29.5 °C whereas in the *valS*ᵗˢ*rel*⁻ strain the rates of accumulation increase to about 60% above the control values. Thus it was concluded that both the accumulation of stable RNA and r-protein are subject to the influence of the stringent control system[7]. The results summarised in Fig. 2 now show further that the stringent control system influences the expression of r-protein genes in these conditions by directly or indirectly regulating the amount of r-protein mRNA available for translation.

RNA, protein and r-protein synthesis

The presence of mRNA for r-proteins in the RNA prepared from the *rel*⁺ and *rel*⁻ strains incubated at the restrictive temperature of 40 °C suggests that r-protein should account for a significant fraction of the residual protein synthesised under these conditions. To verify this we have determined the accumulation rate of RNA and protein and the relative differential synthesis rates of individual r-proteins in these restrictive conditions. The exponential synthesis rates of stable RNA and protein after a shift to 40 °C are illustrated in Fig. 3. In the parental strain the rates of accumulation of stable RNA and protein both increased approximately 1.6–1.7-fold immediately after the shift In contrast protein accumulation almost stopped in both the *valS*ᵗˢ*rel*⁺ and *valS*ᵗˢ*rel*⁻ strains at 40 °C. At the same time stable RNA accumulation was drastically reduced in the *valS*ᵗˢ*rel*⁺ strain and initially increased approximately 1.7-fold in the *valS*ᵗˢ*rel*⁻ strain. These strains thus illustrate the classical stringent and relaxed phenotypes after the inhibition of aminoacylation of valyl-tRNA[3,7].

The relative differential synthesis rates of individual r-proteins[7,11] at the restrictive temperature were determined by prelabelling the strains with ^{14}C-isoleucine at 29.5 °C. The

Table 2 Relative differential synthesis rates of individual r-proteins at the restrictive temperature of 40 °C

r-protein	A_i quotients *valS*⁺*rel*⁺ 29.5 °C	*valS*⁺*rel*⁺ 40 °C	*valS*ᵗˢ*rel*⁺ 29.5 °C	*valS*ᵗˢ*rel*⁺ 40 °C	*valS*ᵗˢ*rel*⁻ 29.5 °C	*valS*ᵗˢ*rel*⁻ 40 °C
S3	0.96	0.90	0.97	0.78	0.97	1.92
S4	0.93	0.89	1.00	0.39	1.01	1.41
S5	0.93	0.89	0.98	1.13	1.01	1.67
S6	1.05	0.97	1.11	0.64	1.18	5.84
S7	0.96	0.86	0.99	0.29	1.05	1.43
S8	0.93	0.84	0.96	0.63	0.97	1.67
S9+S11	0.96	0.91	0.95	0.27	1.02	2.85
S10	0.95	0.93	0.98	0.80	1.01	5.10
S12	0.98	0.73	1.05	0.37	1.10	2.83
S13	0.95	0.87	0.96	0.55	1.03	3.18
S14	0.97	0.80	1.02	1.27	1.04	2.86
S15	0.96	0.87	0.97	0.86	1.02	5.07
S16	0.99	0.93	0.97	0.19	0.99	1.58
S17	0.98	0.90	0.97	0.45	1.01	2.43
S18	0.92	0.80	0.93	0.30	0.98	1.17
S19	0.93	0.89	0.97	0.65	1.01	1.98
S20	0.94	0.93	0.96	0.39	1.00	3.87
S21	1.04	0.99	0.96	0.37	1.08	1.26
L1	0.96	0.89	0.99	0.26	1.02	4.00
L2	0.92	0.94	0.99	0.62	1.05	1.66
L3	0.96	0.85	0.98	0.26	0.98	2.49
L5	0.92	0.87	0.96	1.00	1.00	4.74
L6	0.92	0.90	0.98	0.93	0.96	1.21
L7	0.95	1.02	0.97	0.66	1.05	4.92
L10	0.94	0.90	0.94	0.65	1.00	5.23
L11	0.94	0.89	0.97	0.48	1.01	4.58
L12	0.87	0.76	0.91	0.15	0.97	3.55
L13	0.92	0.88	0.97	0.18	0.99	2.72
L14	0.98	0.91	0.96	1.09	0.93	1.25
L15	0.94	0.93	0.93	0.95	0.97	1.00
L16	0.96	0.97	0.96	1.52	0.96	2.25
L17	0.94	0.94	0.96	0.50	1.00	3.15
L18	0.96	0.89	0.93	1.32	1.02	1.76
L19	0.97	0.94	1.01	0.18	1.03	1.80
L20+S12	1.00	0.98	0.99	0.27	1.09	1.28
L21	0.96	0.93	0.96	0.37	1.00	2.63
L22	0.94	0.92	0.96	1.14	1.06	1.52
L24	0.97	0.94	0.96	0.85	1.02	0.98
L27	0.98	0.92	0.99	1.01	0.98	2.38
L28	1.00	0.93	0.95	0.53	1.08	1.74
L30	0.96	0.97	0.97	2.49	1.01	2.38
L32	0.97	0.95	1.03	1.20	1.01	4.56
L33	0.97	0.96	0.97	2.36	1.06	2.17
L7+L12	—	0.91	—	—	—	—
Mean	0.96	0.90	0.97	0.73	1.01	2.65
s.d.	0.03	0.06	0.03	0.52	0.04	1.36

Bacterial cultures were grown as described in Fig. 1 in medium containing non-radioactive phosphate. At an A_{460} of 0.1, a 10-ml portion of the culture was labelled with ^{14}C-isoleucine (specific activity 306 mCi mmol^{-1}; 3 nmol ml^{-1}). When the A_{460} reached 0.3 the cultures were shifted to 40 °C and after 15 min, labelled with ^{3}H-isoleucine (specific activity 30 Ci mmol^{-1}; 1 nmol ml^{-1}). After 5 min, labelling was terminated by addition of excess isoleucine, leucine and valine (3 μmol ml^{-1}) and incubation continued for a further 5 min and then rapidly cooled to 0 °C. The control samples at 29.5 °C were labelled in a similar manner although the length of the ^{3}H label was reduced to 1 min and the post-labelling incubation period increased to 30 min. Cells were collected and the incorporation of ^{3}H and ^{14}C into total protein was determined as described previously[7,11]; the r-proteins were extracted directly from cell lysates and separated by the gel electrophoresis system of Kaltschmidt and Wittmann[12]. The ^{3}H and ^{14}C radioactivity associated with each of the r-proteins was determined following oxidation and scintillation counting and the A_i values were calculated:

$$A_i = \frac{[^{3}\mathrm{H}/^{14}\mathrm{C}] \text{ in } i^{\mathrm{th}} \text{ r-protein}}{[^{3}\mathrm{H}/^{14}\mathrm{C}] \text{ in total protein}} = \frac{[^{3}\mathrm{H}] \text{ in } i^{\mathrm{th}} \text{ r-protein}/[^{3}\mathrm{H}] \text{ in total protein}}{[^{14}\mathrm{C}] \text{ in } i^{\mathrm{th}} \text{ r-protein}/[^{14}\mathrm{C}] \text{ in total protein}}$$

As the above relationships show, the A_i values give the relative change in the differential synthesis rates of the individual r-proteins after the shift to 40 °C. The control values at 29.5 °C are equal to approximately 1.0. These A_i values are independent of the recoveries of the individual r-protein during extraction and electrophoresis because of normalisation with the ^{14}C prelabel. Furthermore, detection does not require incorporation of the proteins into ribosomal particles since the r-proteins are extracted directly from the whole cell lysates. Detection, however, requires maturation of r-proteins so that their electrophoretic properties are identical to the ^{14}C-labelled r-proteins.

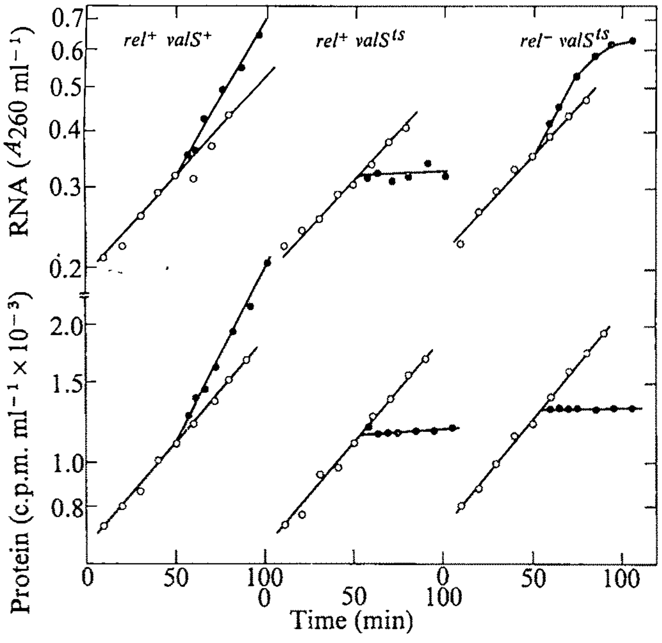

Fig. 3 Accumulation of RNA and protein after a temperature shift from 29.5 to 40 °C. The accumulation of RNA (upper curves) and protein (lower curves) was monitored in the *valS*^{ts}*rel*$^+$ parent (NF314), the *valS*^{ts}*rel*$^+$ strain (NF536) and the *valS*^{ts}*rel*$^-$ strain (NF537) at 29.5 °C (○) and after a shift of a portion of the culture to 40 °C (●). Growth conditions are described in Fig. 1; the growth medium contained 0.2 mM non-radioactive phosphate and radioactive leucine (20 μg ml^{-1}). The temperature shifts occurred at 50 min on the time scales. RNA was measured as A_{260} of acid insoluble, alkali labile material and protein was measured in the three leucine requiring strains as long term ^{14}C-leucine (281 mCi mmol^{-1}, 0.1 μCi ml^{-1}) incorporation into acid insoluble material[7].

cultures were shifted to 40 °C and, after 15 min, were exposed to ^{3}H-isoleucine for 5 min. Incorporation was terminated by addition of excess non-radioactive isoleucine and incubation was continued for a further 5 min to allow time for completion and maturation of nascent r-protein chains and to minimise the possibility of proteolytic degradation of mature ^{3}H-labelled r-protein in the temperature sensitive strains. The [^{3}H/^{14}C] isotope ratios in total cellular protein and 43 separate r-proteins (extracted from whole cell lysates and separated electrophoretically[7,11,12]) were determined and the quotients of the respective isotope ratios were calculated (Table 2). These A_i quotients illustrate the relative change in the differential synthesis rates of the individual r-proteins following the shift from 29.5 to 40 °C.

At the permissive temperature of 29.5 °C, total r-protein synthesis accounts for approximately 13% of the total protein synthesis rate (that is, $\alpha_r = 0.13$)[7]. When the parental strain was shifted to 40 °C, the A_i quotients remained essentially constant and equal to the unshifted control values of approximately 1.0 (Table 2). This indicates that the temperature shift has little or no effect on the balanced synthesis of stable RNA, total protein and individual r-proteins in the non-temperature sensitive parental strain and r-protein continues to account for about 13% of the total protein synthesis rate. This observation correlates with the apparent constancy in the fraction of specific r-protein mRNA in the parental strain (Fig. 2).

In contrast, when the *valS*^{ts} strains were shifted to 40 °C a non-coordinate response of the A_i quotients was observed and indicates an imbalance in the production of individual r-protein species (Table 2). In the *valS*^{ts}*rel*$^+$ strain the differential synthesis rates of some r-proteins increased as much as 2.5-fold (that is, $A_i > 1.0$) while most decreased up to fivefold (that is, $A_i < 1.0$). The average of the 43 A_i quotients was 0.73. Thus, at the restrictive temperature of 40 °C, complete and mature r-proteins account for about 9% of the residual amino acid incorporation although individual r-proteins are synthesised in drastically disproportionate amounts.

In the *valS*^{ts}*rel*$^-$ strain the differential synthesis rates of individual r-proteins increased up to fivefold or more (A_i values between 1.0 and 5.84; Table 2). The average of the 43 individual A_i quotients was 2.65. This indicates that r-protein accounts for about 35% of the residual protein synthesis at 40 °C and again individual r-proteins are produced in drastically disproportionate amounts.

During inhibition of protein synthesis by amino acid deprivation, the chain elongation rate of nascent peptides is reduced and the probability for release of unfinished chains from the ribosome is increased[13,14]. The premature release probability increases with molecular weight and with the content of deprived amino acid, and released fragments are rapidly degraded. Thus, the imbalance observed in the synthesis rates of individual r-proteins at the restrictive temperature may in part result from differences in their molecular weights and valine content. Other processes such as degradation of mature r-proteins which are not incorporated into ribosomal particles[15] or accumulation of precursor forms of r-proteins[11] which escape detection in the electrophoresis system used[12] would also contribute to the non-coordinate production and result in underestimates in synthesis rates of individual r-proteins. In the experimental procedure used here, a 5-min chase following the ^{3}H-isoleucine was provided to enable completion of labelled nascent r-proteins and subsequent maturation and to minimise the degradation of mature r-protein. The possibility remains, however, that at least some of the non-coordinate production of r-proteins may be a direct effect of the mechanism controlling the expression of r-protein genes.

Goodman *et al.*[16,17] previously observed that only one or a few 50S r-proteins accounted for the major portion of the residual protein synthesis in an amino acid deprived *rel*$^-$ strain whereas all r-proteins were produced in nearly proportionate amounts and represented a very small fraction of the residual protein synthesis in a *rel*$^+$ strain. The difference between our results and the earlier work of Goodman *et al.*[16,17] may be explained by the fact that their detection of r-proteins labelled during amino acid deprivation required readdition of the deprived amino acid and subsequent incorporation of the radioactive r-proteins into ribosomal particles. They made no attempt to determine the stability of the free labelled r-proteins and the radioactive proteins associated with the isolated ribosomes were analysed by one-dimensional gel electrophoresis. In our experiments radioactive r-proteins were extracted directly from whole cell lysates within minutes of the termination of the labelling period; incorporation of labelled r-protein into ribosomal particles was not required and labelled r-proteins were separated using the standard two-dimensional electrophoresis system of Kaltschmidt and Wittmann[12].

Regulation of r-protein gene expression

Clear and distinct differences are apparent in the amount of specific r-protein mRNA in *rel*$^+$ and *rel*$^-$ strains during partial or complete inhibition of valyl-tRNA aminoacylation. The physiological significance of these differences is substantiated by the observation that the synthesis rates of rRNA and r-protein remain in balance at semipermissive temperatures up to at least 35.5 °C[7]. That is, during partial inhibition of protein synthesis the expression of rRNA and r-protein genes is coordinately reduced in the *rel*$^+$ strain and coordinately enhanced in the *rel*$^-$ strain.

The synthesis of mRNA in general has been thought to be independent of the stringent control system[5,6]; the amounts of tryptophan[6,16] and β-galactosidase mRNA[18] were observed to be nearly equal in *rel*$^+$ and *rel*$^-$ strains during amino acid deprivation and the half lives of these mRNAs as well as total mRNA were estimated to be essentially identical to the steady state values of 1–2 min[5,6,18,19]. Our results show that the regulation of synthesis of r-protein mRNA is subject to the influence of the stringent control system and is different from that of mRNAs studied previously. In this connection, the experiments of Lazzarini and Winslow[5] should be noted. They observed that the synthesis of total mRNA was reduced to 40% and 65% in the *rel*$^+$ and *rel*$^-$ strains respectively during the initial 20–40 min after amino acid deprivation. At the same time stable RNA accumulation was reduced to about 10% in the *rel*$^+$ and essentially unaffected in the *rel*$^-$ strain whereas residual protein synthesis in both strains was reduced to about 5% of the control rate. Thus, the excess synthesis of total mRNA observed in the *rel*$^-$ strain relative to the *rel*$^+$ strain during amino acid deprivation may reflect to a large extent the increased synthesis of r-protein mRNA we observed (Fig. 2). Furthermore, these observations seem to indicate that the regulation of r-protein mRNA by the stringent control system is exerted at the level of initiation of transcription rather than at the level of mRNA stability. We are investigating this possibility.

We observed that the r-protein mRNA fraction in the *valS*^{ts}*rel*$^+$ strain begins to increase at temperatures above 37 °C (Fig. 2, lower curve). This may be of physiological significance and related to the general mechanism controlling the transcription of r-protein genes. Lazzarini and Winslow[5] observed that the residual stable RNA accumulation (10%) is greater than the residual total protein accumulation (5%) in the *rel*$^+$ strain during amino acid starvation. It is possible that the transcription of r-protein genes is negatively regulated by free r-proteins (that is autogenous regulation[20,21]) such that any rRNA produced in excess during severe amino acid deprivation would deplete the pools of free r-protein and consequently stimulate the transcription of r-protein genes in the *rel*$^+$ strain. This is in fact observed at temperatures above 37 °C. During partial or complete inhibition of protein synthesis in the *rel*$^-$ strain, RNA always accumulates in excess of total protein and the expression of r-protein genes is enhanced (Fig. 2 and ref. 7). Other explanations are also possible, however, and further experiments are required to test the validity of the specific mechanism suggested here.

This work was supported by grants from the US National Science Foundation and the National Institutes of Health. We thank K. Ryan and L. Sadowski for technical assistance.

Received March 10; accepted April 22, 1975

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letters to nature

Lunar occultation of the hard X-ray source in the Crab Nebula

HARD X rays from the Crab Nebula were observed at a lunar occultation on 24 January 1975 with two sets of scintillation counters on board two balloons launched from Hyderabad (17°20′N, 78°27′E). The result obtained by one of the balloon observations is reported here.

The balloon payload comprised eight counters, each consisting of an NaI (Tl) crystal (125 mm diameter × 3 mm thick) with the field of view of 30° cone defined by a cylindrical Pb–Sn collimator with vertical axis. Pulse heights of individual counters were telemetered event by event covering an energy range 20–70 keV. The balloon was floating at the ceiling altitude of 590 Pa (5.9 mbar) when an immersion of the X-ray source took place at about 16 h 43 min UT. The Crab Nebula was located at zenith angle 11°. The Crab pulsar was occultated by the Moon at position angle 102° and at a speed of 0.36″ s^{-1}.

The counting rate averaged for every 10 s is shown in Fig. 1. The transition of the counting rate is clearly observed at about 16 h 43 min UT. The counting rate before the immersion can be represented by a linear relationship $C_1(t) = 284.5 - 0.00533 (t - t_1)$ counts s^{-1}, where t_1 = 16 h 41 min UT and $t < t_1$ is the time (s); the relationship after the immersion is $C_2(t) = 255.9 - 0.00171 (t - t_2)$ counts s^{-1}, where t_2 = 16 h 45 min UT. A decrease before the immersion is associated with a decrease of the elevation angle of the Crab Nebula with time which results in a decrease of the effective area of the counters; the time dependence thereafter is associated with a slow descent of the balloon.

Referring to the standard 10 kHz time signal, we found the apparent period of the Crab pulsar to be 33.17582 ms which is consistent with the barycentric period of 33.1736 ms obtained from the radio observation at Arecibo, as informed by R. Payne. The pulse profile shows two distinct peaks as shown in Fig. 1, and the counts during 3 ms under each peak unambiguously represent the time dependence of the pulsed X-ray counting rate. The counts in every 10 s thus defined for the pulsar component are also shown in Fig. 1. There is a sharp drop at t_p = 16 h 43 min UT, which is slightly shifted from the centre of the transition region in the total counting rate. The counting rate resulting from the total pulsar component (*f*) is obtained from the pulse profile as $C_p = 7.8$ counts s^{-1} which is 26.5% of the total counting rate of the Crab X rays.

Fixing $C_1(t_1)$, $C_2(t_2)$, C_p and t_p, we fitted a Gaussian model of the diffuse source to the observed counting rates between t_1 and t_2. A minimum of $\chi_m{}^2 = 20.8$ (d.f. = 20) is obtained for the centre of the diffuse source t_c = 16 h 42 min 44 s$^{+11}_{-95}$ s UT and the FWHM D_t in units of time is 95 s $^{+48}_{-38}$ s, where the errors indicate the value of one parameter to be $\chi_m{}^2 + 1$

whereas the other is optimised. Note that for $t_c = t_p$ the minimum value of χ^2 is 22.8 for $D_t = 84$ s.

The time profile of the intensity for this model is shown in Fig. 1. The centre of the diffuse source is offset from the pulsar by $d = 6'' \pm 4''$ and the Gaussian width is $D = 34''\,^{+17''}_{-14''}$. The former is as large as the angular distance between the pulsar and Wisp 1, whereas the latter contains the pulsar, four Wisps and Anvil, as shown in Fig. 2.

The observed data were also fitted to an alternative model that assumes two essentially line sources and the pulsar in between. A minimum of $\chi_m^2 = 17.2$ is obtained for the relative intensities of these sources and their positions: 48% (16 h 42 min 30 s), 26.5% (16 h 43 min 00 s = pulsar), 25.5% (16 h 43 min 20 s). The first line source is located on the Wisps, whereas another is on the Anvil on the other side of the pulsar.

The average energy of hard X rays (~50 keV) and the position angle of 102° found are close to the situation for the immersion in the MIT experiment[1]. Our result is compared with their Fit $2f = 25 \pm 16\%$, $d = 11'' \pm 4''$ and $D = 23'' \pm 6''$ at p.a. 130°. The size of the diffuse source is significantly smaller than that found in the keV region [2-4]. Further detail will be described elsewhere.

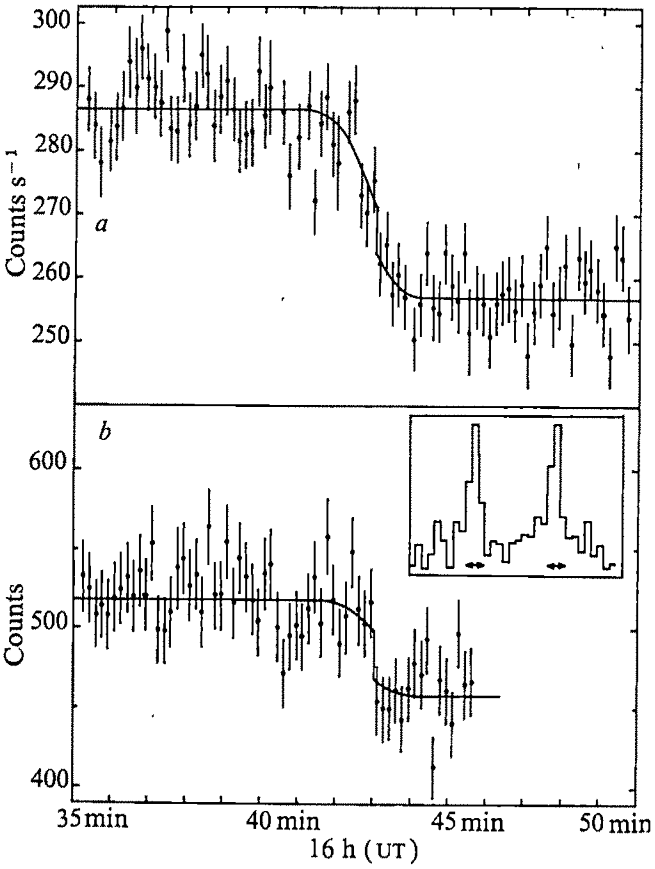

Fig. 1 The total counting rate (*a*) and the counts in 10 s bins for the pulsation peaks as indicated by arrows in the insert (*b*). The solid curve in the upper figure represents the total counting rate expected for the Gaussian diffuse source of FWHM of 95 s and centroid displaced by 16 s from a point source. The contribution of the point source (pulsar) relative to the total Crab X rays is 26.5%, judged from the pulse profile assuming no steady emission from the pulsar, and the contribution of the pulsation peaks as indicated in the insert is 51% of the total emission. The solid curve in the lower figure represents the counts in 10 s bins with phase corresponding to the peaks.

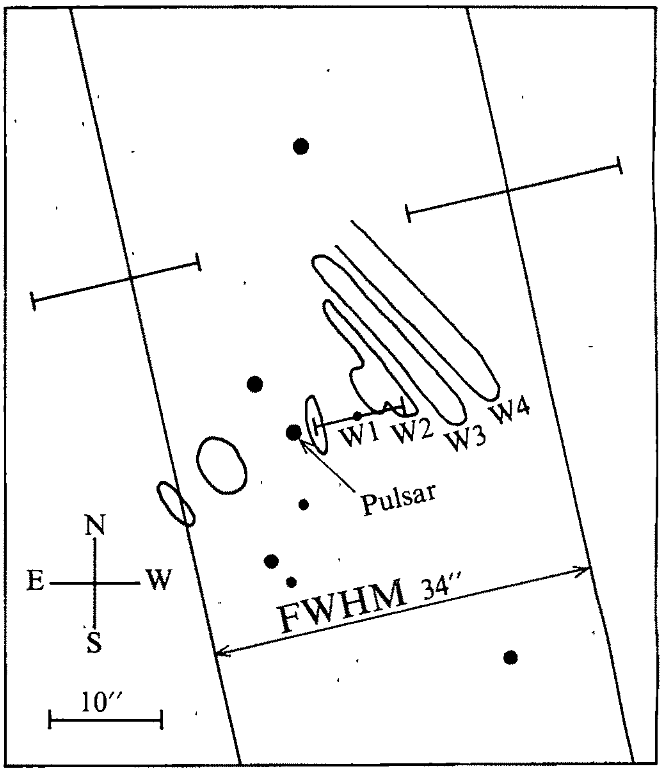

Fig. 2 X ray emitting region superimposed on the optical features of the Crab nebula given by Scargle and Pacini[5]. Wisps are indicated by W1, · · · , W4; the Anvil is represented by two bright features on the east side of the pulsar. The centroid of the Gaussian diffuse source and FWHM, with their error bars, are also shown. Note that the error bars for the boundaries of the diffuse source are obtained from $\chi_m^2 + 1$ for parameters $t_c \pm D_t/2$.

We thank the staff of the Balloon Facility, Tata Institute of Fundamental Research for launch support. Occultation data were supplied by Mr L. V. Morrison of the RGO and Dr A. M. Shinzi and Mr K. Inoue of the Hydrographic Department of Japan. Grants from the Mitsubishi Foundation, Nissan Science Foundation and Ministry of Education of Japan are acknowledged.

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Received April 14, 1975.

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Variability and circular polarisation in the nucleus of NGC5128 at 10.7 GHz

THE galaxy NGC5128 (Centaurus A), has been found to contain a compact radio source in its nucleus[1]. This compact source is one of the most remarkable yet discovered, and, as NGC5128 is the nearest of the strong radio galaxies (~4 Mpc), it provides a possibly unique opportunity to investigate the physical processes which produce these objects. The results which we report here place important constraints on its structure and magnetic field strength.

We have reported[2] that the 10.7-GHz flux density of NGC 5128 is substantially greater than those previously measured[1] at 2.7 and 8.1 GHz. If the radio spectrum is interpreted as arising from a single optically thick source of synchrotron radiation, then the high turnover frequency plus a lower limit[3] of 0.001 arc s on the angular size at 2.3 GHz implies the presence of a strong magnetic field ($\geqslant 10$ gauss). Recently 31.5 and 89 GHz flux densities several times greater than the flux we measured at 10.7 GHz have been reported[4]; at these frequencies the source seems to vary by ~50% on a time scale of a day or less. The source has also been detected at 1.4 GHz (J. O'Sullivan, unpublished) and an upper limit has been placed on its flux at 0.4 GHz (B. Mills, unpublished); the radio flux densities including our results are summarised in Table 1. Centaurus A is a source of X rays[5,6] which are believed to originate in the nuclear region[7]. Outside the nucleus it is probably[8] an emitter of very high energy gamma rays ($h\nu > 10^{11}$ eV).

The traditional interpretation of compact radio sources is that clouds of relativistic electrons radiate by the synchrotron mechanism. Any homogeneous synchrotron source becomes optically thick below some frequency ν_{max} where the flux density is S_{max}. The magnetic field, B, and angular diameter, θ, are related by[9]

$$B = 1.3 \times 10^{-36}\, \theta^4\, \nu_{max}{}^5\, S_{max}{}^{-2} \qquad (1)$$

where B is in gauss, θ in arc s, ν_{max} in Hz, and S_{max} in Jy. It is expected that there will also be high frequency emission from the relativistic particles by either the synchrotron or inverse Compton mechanisms or both, and this is proportional to $\theta^{-2}B^{-(1+\alpha)}$ where α is the high frequency spectral index[10]. So it seems possible to determine both angular diameter and magnetic field strength from combined observations of a source's spectrum at radio, optical, X-ray and gamma-ray wavelengths[10].

Table 1 Radio fluxes for the nucleus of NGC5128

Frequency (GHz)	Flux density (Jy)
0.4	<20
1.4	5.0
2.7	2.4
8.1	2.9
10.7	4–8 (variable)
31.5	20–25
89	15–25 (variable)

But it is also possible to measure angular diameters directly by very long baseline interferometry, and the degree of circular polarisation provides an independent measure of the magnetic field strength; it is proportional to $(\nu^{-1} B \sin\varphi)^{1/2} \cot\varphi$ where ν is the observing frequency and φ the angle between the magnetic field and the line of sight. Furthermore, if radiation at two different frequencies arises from the same ensemble of relativistic particles, the time scales for any variability observed at those frequencies should be the same in the optically thin regime; if the component is optically thick, however, they are related in a way which depends on the detailed operation of the mechanism which produces the variability.

Table 2 10.7-GHz time variations in the nucleus of NGC5128

Date	10.7-GHz flux density (Jy)
June–July 1973	4.4
May 1974	8.1
January 1975	7.4

We have observed the nucleus of NGC5128 in May 1974, and on two consecutive nights in January 1975. All observations were made with the Stanford University five element radio telescope[11]. An attempt to detect circular polarisation was made during the January 1975 observations; in May 1974 only the 10.7-GHz flux density was measured.

The nucleus of NGC5128 is probably variable at 10.7 GHz; however, the time scale for this variability may be significantly longer than that reported at millimetre wavelengths[4]. Although we must observe Centaurus A (declination−43°) at elevations just above the horizon, its nature is such that we can use the Stanford interferometer to make relative measurements of the flux density of the compact central component from one epoch to another free from any systematic errors that might arise because of calibration difficulties. This is because the inner radio lobes of Centaurus A fall partially within our field of view, and we are able to compare the measured intensity of the central core to that of selected points in those lobes both to its east and west. The central source increased in strength by the same amount relative to the selected points on its opposite sides, some 85% (~8σ), between July 1973 and May 1974, but changed little between then and January 1975 (Table 2). The relative intensities of the points in the lobes, however, remained constant to within 15% (1.5σ) between the various dates in question. It would be difficult to explain such a large intensity change affecting only the compact central source by an instrumental effect. On the other hand, we observed the source on six days in 1973 and on two consecutive nights in 1975 and found no evidence for intensity changes of the order of 50% from one day to the next, as reported at millimetre wavelengths. So it seems that the time scale of variability in NGC5128 is significantly different at 10.7 GHz from that at frequencies between 30 and 90 GHz.

We failed to detect any circularly polarised flux and from the fluctuations of the measured crossed-horn visibilities we calculate a 5σ upper limit of 2.5% to the fraction which might be present.

Our circular polarisation limit probably allows us to rule out one-component models for the nucleus and place important constraints on two-components models. When source variability is considered, the 1.4-GHz flux density might be reconciled with the spectrum of a single source, opaque below 30 GHz; however, the magnetic field in such a source would be ~50 gauss and the resulting degree of circular polarisation at 10.7 GHz would be 25% for a field making an angle of 45° to the line of sight. Grindlay[10] has proposed a model in which all the electromagnetic radiation emitted by the nucleus arises from two Compton–synchrotron emitting components, one opaque below 1 GHz with $\theta = 9 \times 10^{-3}$ arc s and $B = 0.01$ gauss, the other opaque below 30 GHz with $\theta = 4 \times 10^{-4}$ arc s and $B = 2$ gauss. In this model the smaller component dominates the emission at 10.7 GHz and requires 5% circular polarisation there if the angle between the magnetic field and line of sight is again 45°. Alternatively, such a component with a 2-gauss field can be reconciled with our limit on the degree of 10.7-GHz circular polarisation only if that angle is 10° or less. But the precise values of magnetic field and angular diameter in Grindlay's model are sensitive to the turnover frequencies assumed and the slope of the spectrum between 10^{19} and 10^{26} Hz. These are poorly determined by the data which, within their uncertainty, could allow a two-component structure with magnetic field somewhat less than 1 gauss for the more compact component, and this could be reconciled with our circular polarisation limit.

Our limit on circular polarisation is not sufficiently stringent even to rule out the highly idealised model of Grindlay[10], given the uncertainties in the other observed parameters. Condon and Dressel[12] have discussed models in which there is a radial change in the particle density and field strength and radio variability at low frequencies is caused by expanding shock fronts. Since the accuracy of our circular polarisation measurement can be improved by an order of magnitude, and repeated at other frequencies, it would be valuable to fit such models to the spectrum of NGC5128 and calculate in detail the expected degree of circular (and linear) polarisation as a function of frequency.

We thank Dr W. Medd and Dr G. A. Harvey of the National Research Council of Canada for supplying us with monthly 10.7-GHz flux densities of variable calibrator sources. The Stanford five element interferometer was built with support from the Air Force Office of Scientific Research supplemented by NSF grants. Observations and analysis were funded by the Electrical Engineering Department of Stanford University.

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Received February 18; accepted April 30, 1975.

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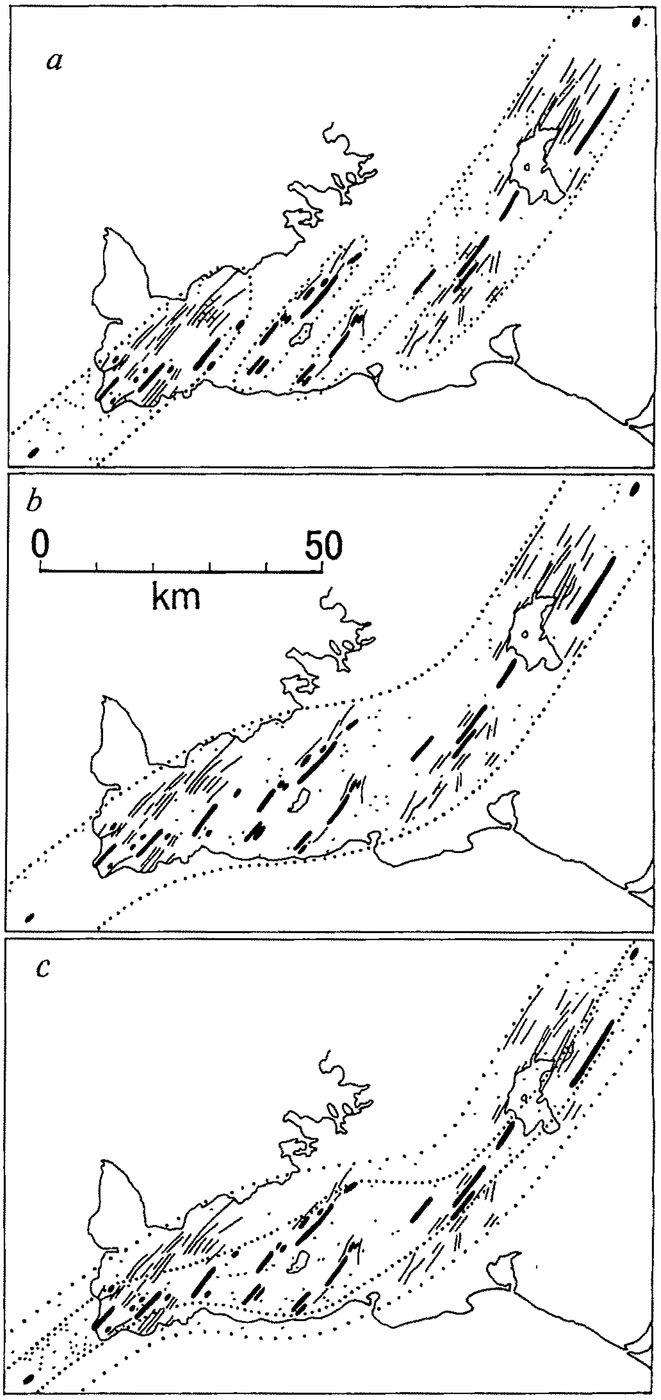
Excess spreading axes and spreading rate in Iceland

Rifting and volcanic activity in Iceland is believed to be related to two active rift zones. I suggest, however, that several additional zones, some having a limited life, also exist. This concurs with a view[1] that the active zones are not immutable. It is now becoming increasingly apparent, moreover, that the southern half of Iceland has a spreading rate several times greater than that of both the northern half and some submerged parts of the Mid-Atlantic Ridge.

The term 'axial rift zone' is applied here to a zone of crustal spreading in which fissures (both eruptive and non-eruptive) are surface expressions of the rifting and are accompanied by axial subsidence. Axial rift zones are further classed as 'active' if they contain postglacial fissures or eruptive centres (active within about the past 10 kyr), 'dormant' if they lack postglacial fissures but contain young moberg (intraglacial hyaloclastite and pillow lava) mountains or young interglacial volcanoes formed towards the end of the Ice Age, and 'extinct' if they lack young, constructional volcanic features. An extinct rift zone is marked by a dyke swarm along the axis of a syncline in which the dykes are the sub-surface expression of both the eruptive and non-eruptive fissures.

The active rift zones can be delineated in different ways (Fig. 1*a* and *b*) according to the scale on which the fissure distribution is examined. In Fig. 1*a* the edges of active zones have been drawn near the outermost postglacial fissures and the continuity of zones is extrapolated across areas where fissures may be concealed by ice, water, or recent sedimentary and volcanic accumulations. Figure 1*b* shows a broader interpretation and may perhaps approach more closely the fundamental underlying structure (Fig. 1*c*). There is a narrow median zone within which magma is generated and erupts at the surface, and narrow marginal zones which have fissures but in which magma fails to reach the surface. It may be that

Fig. 1 Fissure distribution in south-western Iceland; *a* and *b*, two differing views of the configuration of the rift zone (stippling, active rift zone; thick lines, eruptive fissures and vents; thin lines, non-eruptive fissures); *c*, a median zone (close stippled) in which magma reaches the surface, and marginal zones (open stippled) in which it does not. Interpretation *a* is believed to express most satisfactorily the structural relationships, and *c* the thermal relationships, in the rift zone.



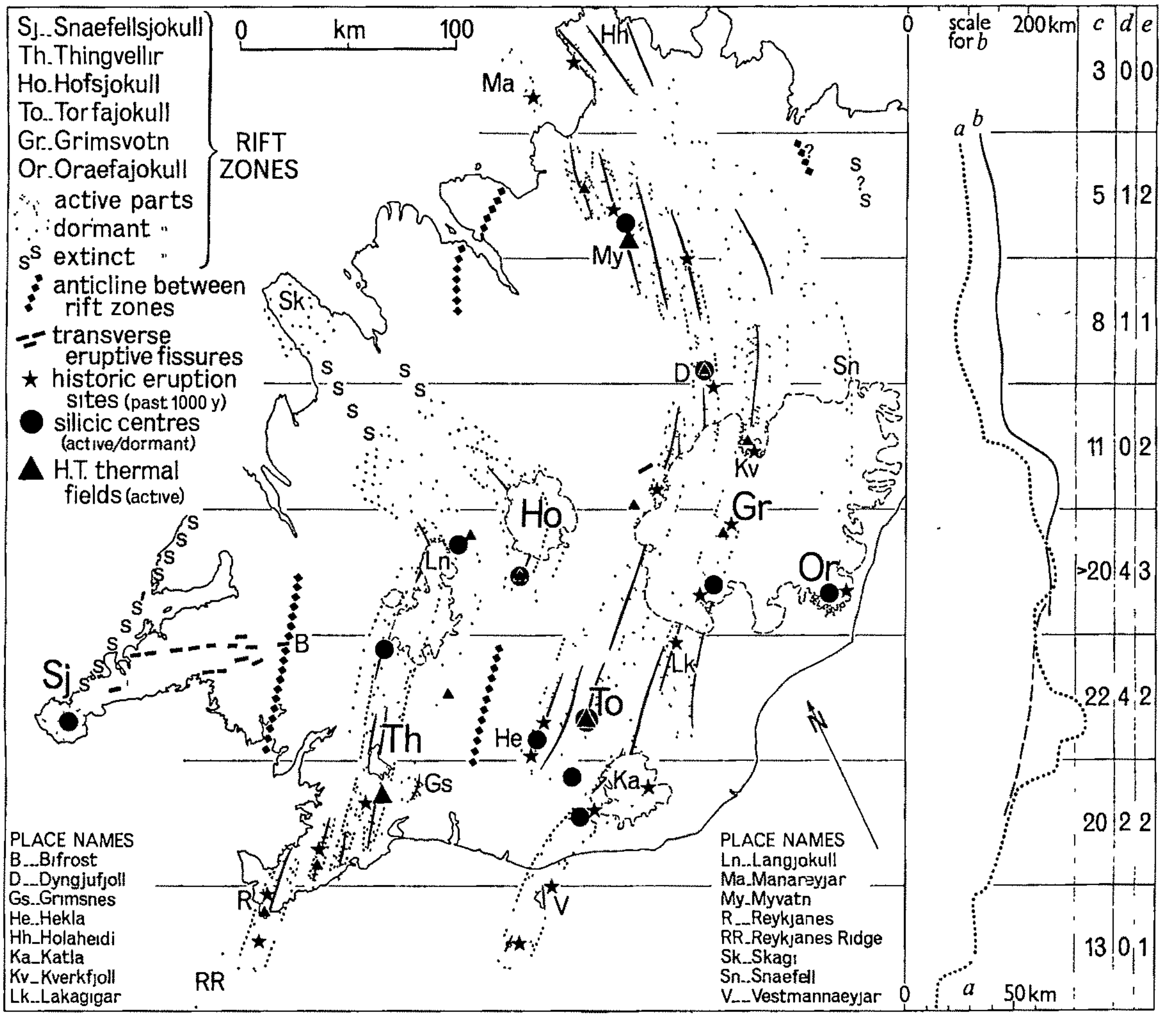


Fig. 2 The distribution of rift zones in Iceland. Curve *a*, aggregate width of active rift zones; *b*, aggregate width of outcrop of rocks younger than 3 Myr old; *c*, number of recorded eruptions in the past 1,000 yr; *d*, number of active and dormant silicic volcanic centres; *e*, number of active high temperature thermal fields. (In every case the number is taken for each of the eight strips drawn across Iceland in the presumed spreading direction.)

magma is generated only in the median zone and when it moves sideways into the marginal zones it fails to reach the surface there because of its lower volumetric rate of rising, caused by sideways flow.

The separate areas of fissures in south-western Iceland (Fig. 1*a*) are regarded as offset sectors of a single rift zone arranged *en echelon* and related to a continuous median zone of magma generation at depth. The offset sectors themselves trend parallel to the fissure direction. I do not accept the view that the sectors have been offset by north-westerly trending faults[2]. A similar but less clear *en echelon* arrangement is seen in the Myvatn–Manareyjar area (Fig. 2). It is supposed that the amount of spreading in a given time is constant along any line drawn across the *en echelon* sectors in the spreading direction.

I suggest that the active zone around Hekla and Myvatn (Fig. 2), previously regarded as a single broad zone, in fact comprises a pair of narrow, active rift zones separated by a 20-km strip within which there is no postglacial activity. The eastern zone of the pair is the Grimsvotn Zone (Fig. 2) which is dormant in its northern part although it is believed to extend to Holaheidi where, for a stretch, it is again active. The western zone of this pair is the Torfajokull Zone (Fig. 2) which is active along its entire length, with *en echelon* offsets both south and north of Dyngjufjoll. In places, this zone may itself be doubled, notably at Hekla, but also at Myvatn.

Another proposed rift zone is the Oraefajokull Zone (Fig. 2) which is dormant along most of its length and is active only at its southern end. Young moberg mountains occur at, and north of, Snaefell, and intraglacial eruptions beneath the ice sheet between Oraefajokull and Snaefell have given rise to the hyaloclastic lava flows which formed under some valley glaciers in south-eastern Iceland[3]. The dormant northern continuations of the Grimsvotn and Oraefajokull zones have been broadly delineated (Fig. 1*b*) pending more detailed studies of the young moberg mountains in this area.

The Thingvellir Zone (Fig. 2) has a sharp inflection at Langjokull[4], and the northward continuation to Skagi is dormant or extinct. The Hofsjokull Zone seems to be separate, with a dormant or extinct northward continuation towards Skagi. The final rift zone is that of Snaefellsjokull (Fig. 2), active only at its south-western end and extinct elsewhere.

The aggregate width of these active zones, measured across Iceland along parallel lines trending in the presumed spreading direction (Fig. 2*a*), reaches a maximum of about 80 km in the southern half of Iceland and falls to about 25 km N from Kverkfjoll; it also falls off to some extent in the coastal belt of south-western Iceland.

I postulate that the spreading rate is more or less proportional to the aggregate width of the active zones, reaching a maximum in the southern half of Iceland, where it is roughly three or four times greater than that in the northern half (which, in turn, is presumably similar to the rate in submerged parts of the Mid-Atlantic Ridge). This is supported by several lines of evidence. First, the total width of outcrop of rocks younger than 3 Myr (formed since

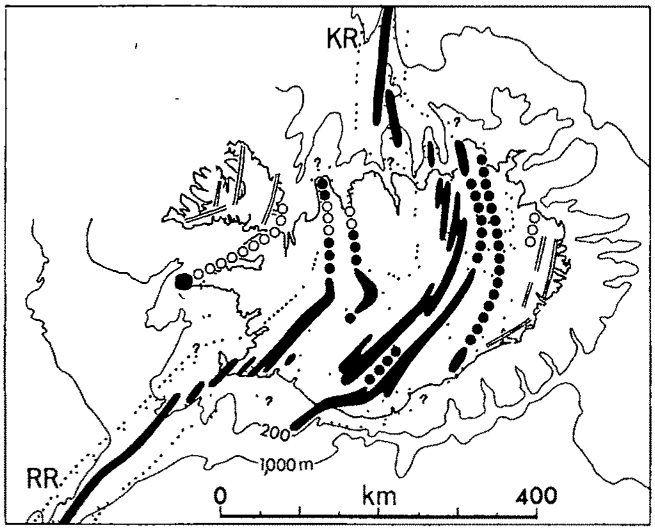


Fig. 3 Summary map of the distribution of rift zones in Iceland and their relationship to the spreading axis along the Mid-Atlantic Ridge axis. KR, Kolbeinsey Ridge[17]; RR, Reykjanes Ridge[14,18]; solid areas, active rift zones; ●, dormant rift zones; ○, extinct rift zones; stippling, crust less than 3 Myr old; ═══, Tertiary dyke trends. The active zones tend to be parallel with prevalent dyke trends in the Tertiary basalts of eastern[12] and north-western Iceland[19].

the Mammoth Event of the Gauss geomagnetic epoch) reaches a maximum of 250 km in southern Iceland[5] compared with 140 km in northern Iceland (Fig. 2*b*) and 60 km in the submerged part of the Mid-Atlantic Ridge[6] (Fig. 3). The outcrop width on land is admittedly inflated by the overlap of younger lavas on to older lavas, but overlap cannot account for the gross difference in width within Iceland. Second, the frequency of volcanic eruptions (those of the past 1,000 yr are included, Fig. 2*c*)[7] for strips of equal width trending in the spreading direction, is several times greater in southern Iceland than elsewhere; the number is taken to be a direct function of the spreading rate. Third, the number of active and dormant silicic volcanic centres is greater in southern Iceland, and by far the biggest centre (Torfajokull) occurs in the south (Fig. 2*d*); the number and size is taken to be a direct function of the intensity of basaltic magmatism[8]. Finally, the number of active high temperature geothermal fields is greater in southern Iceland than elsewhere (Fig. 2*e*); it is taken to be a direct function of the intensity of magmatism.

The total discharge pattern of volcanic rock in postglacial time, previously regarded as reaching a maximum in middle Iceland[9], in fact conforms more closely to the southern Iceland maximum.

Adjustment to varied spreading rates occurs by faulting along transverse fracture zones[10]. The two most seismic zones are those between Thingvellir and Katla and west of Manareyjar, with the related Tjornes Fracture Zone[11] on land. The scattered postglacial eruptive vents, developed along fissures transverse to the normal fissure trend between Snaefellsjokull and Bifrost, are thought to be related to such adjustments[10], as is the single transverse eruptive fissure east of Hofsjokull.

The locus of maximum spreading is at present situated in the southern half of Iceland. It has been suggested[12] that 10–15 Myr ago the locus was situated father north where it gave rise to the eastern Iceland Tertiary volcanic pile with its numerous silicic volcanic centres and fossil, high temperature, geothermal fields; it was situated further south 5–10 Myr ago, when the south-eastern Iceland lava pile accumulated with its silicic centres, fossil geothermal fields, and intrusions of gabbro and granophyre; and it has since migrated further south still. The intensity of volcanism at the locus may also have increased with time.

All of the active zones were active in late moberg times except the part of the Grimsvotn Zone around Katla and Lakagigar, which seems to have become active in the past 10–100 kyr, and the northern sections of several zones were active in late moberg times and have become dormant in the past 10–100 kyr. This suggests that the active zones are transitory and some may have a life of only 1 Myr.

The 20 km strip between Torfajokull and Katla is a dormant zone of young moberg mountains which raises the interesting possibility that the Grimsvotn Zone has moved eastwards, perhaps after being 'spawned off' from the Torfajokull Zone. The double sections of the western zone at Hekla and Myvatn could even be places where 'spawning' is in progress, as could the Thingvellir–Grimsnes pair. These double zones are, however, more analogous to the pair of parallel dyke swarms, 5 km apart, of the Breiddalur and Thingmuli volcanic centres in eastern Iceland[13]. The period of formation of these centres and of their dyke swarms overlapped, and although subsidence affected the core of each centre no true anticline developed between them. In contrast, an anticline does develop between separate rift zones, as between Thingvellir and Hekla. The difference is, however, one of degree rather than of kind.

The line of the median rift of the Mid-Atlantic Ridge (KR–RR, Fig. 3) has an inflection at Iceland. Where it crosses Iceland this line is dormant or extinct along part of its length and active spreading occurs in part from rift zones lying farther east. One can speculate that new rift zones are generated from time to time, mostly on the convex side of the inflection where expansion can proceed more readily, and most of the excess spreading in Iceland is accommodated by the creation of new crust on that side. The Tertiary basalts of eastern, Iceland may have been rotated and moved northwards by the subsequent creation of excess crust in the southern half of Iceland so that they are now no longer directly opposite the outcrops of similar age in western Iceland.

The prevalent view that the Iceland fissures are purely dilatational and that the magma plays a passive role and rises along them "by invitation" has been questioned[14], and a compressional regime has been indicated by borehole strainmeter measurements[15] and by an analysis of the 1973 Heimaey fissures[16]. My view that the spreading rate is several times greater in part of Iceland than elsewhere on the Mid-Atlantic Ridge implies that magma plays a very active role in creating and jacking open the fissures there and, indeed, perhaps in causing crustal spreading to occur.

It has often been thought that Iceland offers a unique opportunity to study the details of the spreading process as it operates underwater in a mid-oceanic setting. Iceland is, however, anomalous in that it stands above sea level. I postulate that it is also anomalous for two additional reasons: it has six active rift zones in place of the one believed to exist in submerged sections, and it has a spreading rate which in southern Iceland is several times greater than on submerged parts of the ridge. The question of whether these additional anomalies stem from the fact that Iceland stands above water, or whether Iceland stands above water because of these anomalies, is left open.

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Received March 17, accepted April 29, 1975.

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Significance of calcium-rich differentiates in chondritic meteorites

KNOWLEDGE of conditions in the early Solar System is based largely on results from the study of meteorites. We have found in two chondritic meteorites 'chondrules' or inclusions containing material with the composition of a calcic plagioclase. We believe that this may be evidence of differentiation on a planetary body before compaction of the chondritic parent body(ies) occurred (see also ref. 1).

The Bovedy Meteorite fell in Northern Ireland in 1969 (refs 2 and 3) and is an unequilibrated ordinary chondrite of the low iron group (L3 in the classification of Van Schmus and Wood[4]). Microprobe analysis of the meteorite showed that one large chondrule, 4 mm in diameter, comprises phenocrysts of low calcium–pyroxene (Fs$_{17-28}$) with included grains of olivine (Fa$_{24}$) and a clear isotropic interstitial phase with the composition of a plagioclase (An$_{85}$). Part of a large bleb, approximately 2 mm across, occurring on another specimen of the meteorite also has the composition of plagioclase (An$_{83-88}$): an X-ray powder photograph showed no plagioclase lines, indicating that this bleb is mainly glass. It does, however, contain minor whitlockite and an opaque mineral, probably chromite, though these did not show up on the X-ray photograph but were observed during the microprobe work. A 2.55-mg sample of this bleb was analysed for the rare earth elements by an instrumental neutron activation technique[5] and was found to have a remarkably high, positive europium anomaly. The low abundances of rare earth elements and the magnitude of the europium anomaly are similar to those observed in lunar anorthosites[6,7] but differ from those of the plagioclases in both the eucritic meteorites[8,9] and the lunar mare basalts[10] (see Table 1).

The second chondritic meteorite, Goodland, was found in Kansas in 1923 and is classified in the same low iron group as Bovedy but it is petrographically a type 4[4] and is almost equilibrated[11]. A chondrule in this stone contains some slightly birefringent material with the composition of a plagioclase (An$_{85}$) interstitial to crystals of olivine (Fa$_{25}$) enclosed in orthopyroxene (Fs$_{16}$).

By analogy with the lunar surface, the calcic plagioclase material of Bovedy probably formed by igneous differentiation on a planetary scale. The same could be true of the similar material in Goodland but the absence of data on the rare earth elements makes confirmation of this impossible. Accepting this, it is likely that at least one planetary body had been differentiated and partially fragmented so that its disrupted material was incorporated into an L-group, chondritic, parent body. Goodland has a short gas retention age which precludes speculation about its early history[12]; the age of Bovedy is currently under investigation in the hope that it will provide a younger limit to the time of differentiation of the parent of its calcic feldspar glasses.

Table 1 Rare earth element concentrations (p.p.m.) in Bovedy glass compared with those of lunar and meteoritic samples[6-10]

	Bovedy glass	Lunar anorthosites, bulk data (average of 10)	Plagioclases from lunar mare basalts (average of 12)	Plagioclases from eucrites (average of 3)
Lanthanum	0.25	0.26	7.2	—
Samarium	0.12	0.084	2.4	0.55
Europium	1.35	0.95	3.2	1.1
Ytterbium	0.06	0.051	1.6	0.23
Lutetium	0.0068	0.0085	0.22	—
Europium anomaly	+33	+31	+3.6	+6.0

There seem to have been two distinct periods of heating among planetary bodies in the early Solar System. The first caused the melting and differentiation which led to the production of calcic plagioclase on a planetary body. Portions of this were subsequently available for incorporation into the chondritic parent body which was at this time still relatively undifferentiated. The second heating event caused the metamorphism[4,11] of at least one (L-group) ordinary chondrite parent body. Differences in the initial ^{87}Sr/^{86}Sr ratios between the metamorphosed (H6) chondrite Guarena and the eucrites are also indicative of the occurrence of two heating events separated in time[13].

Four general sources of energy have been proposed to account for these heating events, namely: the release of accretional energy, the decay of short lived radionuclides, the decay of long lived radionuclides, and intense solar radiation[14]. At least two of these are required to account for the two heating cycles outlined above, as each could have been effective once only. Whichever combination of heat sources was responsible for these heating events, evidence for the earlier should best be seen in the least metamorphosed chondrites. It is in these meteorites that the products of the earlier planetary differentiation should be best preserved. We suggest that this preservation has occurred and can be seen in the calcium-rich glass in the Bovedy Meteorite and also, though less certainly, in the calcium-rich material of the chondrite Goodland.

We thank D. Y. Jérome and J. C. Philippot for the neutron activation analysis, and I. G. Meighan for providing us with the Bovedy sample in which he pointed out the glassy bleb (now BM 1972,233).

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Received February 28; accepted April 28, 1975.

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Pyrite–haematite alteration as a source of colour in red beds and regolith

THE colour in red beds and red soils is generally acknowledged to be caused by the presence of finely dispersed haematite or hydro-haematite. The origin of the haematite remains, however, in dispute[1-3]. In the case of present-day red weathering in the tropics and subtropics, the haematite or other colouring material is obviously derived by *in situ* alteration of iron-bearing minerals in the parent rock or soil, but opinion is divided as to whether the haematite or its precursor are of detrital or authigenic origin[2,3] and Chukhrov[1] considers that red beds could never have formed from laterite. The minerals thought to have altered to give iron oxides and hydroxides are silicates, particularly olivine, pyroxene, biotite and hornblende, and the oxides magnetite and ilmenite. I describe here a case of alteration of pyrite to haematite with the subsequent development of red regolith, and suggest that the alteration of detrital or biogenic pyrite may have more general application to the problem of red beds.

Red-weathered regolith (Munsell colours 5-10R 4-6/5-10) is widespread in the Wellington area of New Zealand[5]. It is considered to have formed in the Pleistocene, during warm interglacial periods when the average annual temperature was at least 3 °C higher than at present, and there was a pronounced dry season. At least three periods of red-weathering are represented in the Wellington area. Near Upper Hutt, 40 km north-east of Wellington, red-weathered horizons underlie and overlie the brown-weathered, rhyolitic Mangaroa Ash[6]. This ash has been dated as mid to late Pleistocene (M. T. Te Punga, personal communication).

During examination of the heavy mineral content of the Mangaroa Ash, I noted numerous small cubes and spherules of haematite. The perfect geometry of the cubes suggested that the haematite was pseudomorphous, and electron probe microanalysis of some of the hardest grains showed them to have a core of iron sulphide of composition corresponding to pyrite or marcasite. In contrast to the almost complete alteration of the pyrite, magnetite and the scarce ferromagnesian silicates were almost completely unaltered. The brown colour of the ash derived from rather weathered brown volcanic glass which was the main constituent and the haematite pseudomorphs did not contribute much to the general colour. In the overlying red-weathered, loess-like material, however, cubes and spherules of haematite occur in all stages of disintegration, and finely dispersed haematite gives the deposit a bright, brick-red colour. Magnetite and ferromagnesian silicates in the loess are relatively unaltered.

Some indication of the conditions in which pyrite alters to haematite is given by the recent or present day alteration of pyrite concretions in Israel[7], and in Northland, New Zealand. The Israeli concretions are found in an Upper Campanian to Palaeocene calcareous sequence in a particularly arid area of the Negev. Alteration has produced haematite and goethite on the outer parts of the concretions, whereas the inner parts contain jarosite, gypsum, baryte, celestine and sideronatrite. The alteration of the concretions is thought to be very recent, and the preservation of sideronatrite is considered to be a result of the aridity in the area. The fact that the iron oxides and hydroxides are restricted to the outer parts of the concretions is not surprising: phase diagrams[8] indicate that iron oxides form with increasing *p*H. In this case the formation of iron oxide is probably influenced by the enclosing calcareous beds. The mean annual temperature in the Negev is about 20 °C. In Northland, New Zealand, pyrite nodules on the beach at Whangaroa are altered to haematite on some surfaces. Whangaroa lies at latitude 35°S, the mean annual temperature is 15.5 °C, and the rainfall is 1,780 mm per annum with a dry season in summer, when temperatures may reach 30 °C. Small areas of laterite have formed on volcanic deposits in the area. In contrast, Upper Hutt has a mean annual temperature of 12.5 °C, and a rainfall of 1,270 mm evenly distributed throughout the year. Haematite is not forming in soils of the Wellington region at present, and the red-weathering is considered to be relict[5].

The presence of spherules of haematite suggests that biogenic pyrite has been altered in both the ash and overlying loess. I have noted similar pseudomorphs, both cubes and spherules, from Mangaroa Ash at other localities in the Wellington area, and in view of the widespread occurrence of pyrite in sedimentary rocks, pyrite–haematite alteration may be a source of red colour in beds elsewhere. It is perhaps significant that many red beds and red palaeosols are found in environments where pyrite may be expected to be abundant, such as coal measures, lagoons and shallow inland seas[2]. But although magnetite is frequently reported from red beds formed in these environments, I can find few records of pyrite. This suggests that the pyrite may have been altered; the instability of pyrite in oxidising conditions is extremely well known.

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Received March 25, accepted April 21, 1975.

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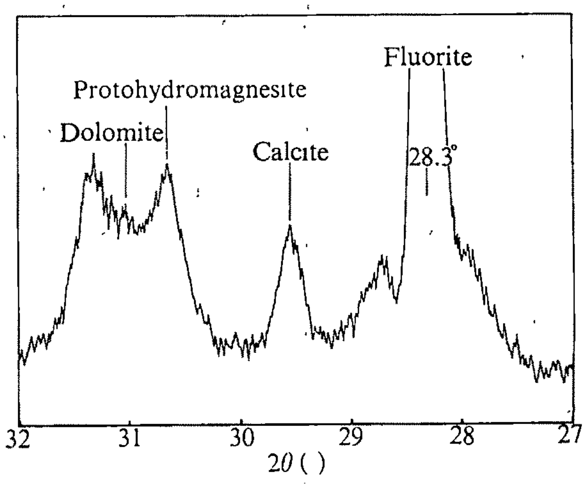
Dolomite and organic material

GEBELEIN and Hoffman[1] drew attention to the association of dolomite with stromatolitic sequences, and suggested that the precipitation of dolomite is related to the presence of organic material. Their experiments confirmed previous results[2,3] that large quantities of magnesium may be absorbed from seawater by organic material. Magnesium-rich algal filaments may form sites for the precipitation of high magnesium calcite with 17–20 mol % $MgCO_3$ and although dolomite has not been precipitated in such conditions, it is possible that high-Mg calcite could be diagenetically altered to dolomite because of the high Mg–Ca ratio associated with the organic material[1].

We report here on experimental and field studies, and consider the experimental data of Gebelein and Hoffman in conjunction with other data from localities at which dolomite is precipitated.

In a simulation experiment performed in a large tank[4], a shallow water, organically rich, evaporitic carbonate environment was simulated and allowed to evolve over a period of eight to nine months. Mineralogical, chemical, and biological parameters of the supernatant and sediment environment were

Fig. 1 X-ray diffraction diagram of sample of decaying filamentous algae sandwiched between calcite and nesquehonite layers.



monitored regularly. An important feature of this experiment was that a layer of decaying filamentous algae was placed between layers of crushed calcite and nesquehonite. At the end of nine months, samples of the decaying filamentous algae were collected, washed, dried, and X rayed (Fig. 1). Dolomite had precipitated within the organic material. This established for the first time that a causal relationship exists between dolomite and organic material. Lippman[5] showed that dolomite did not precipitate from purely inorganic mixtures of calcite and nesquehonite, a fact verified within our laboratory. The X-ray diagram (Fig. 1) shows in addition that protohydromagnesite[6], and possibly huntite, also precipitated within the organic material.

One of us (P. J. D.) has studied One Tree Reef, in the southern Great Barrier Reef. In the middle of the rubble cay at the southern end of the reef is a small semipermanent pool, in which carbonates are being precipitated. The sedimentary section within the pool is shown in Fig. 2. An algal mat (Fig. 2*a*) approximately 3–5 mm thick covers the surface, except near the edge of the pool where land snails are grazing. Below the algal mat is a spongy pale pink to brown organic layer (Fig. 2*b*) which may be up to 75–100 mm thick. This rests on a white precipitate of aragonite and high magnesium calcite (Fig. 2*c*). Samples of the organic material were vacuum dried and X rayed (Fig. 2). Dolomite occurs within the organic material, which corroborates the laboratory experiments. It is pertinent also that no dolomite is found in any of the carbonates below the organic layer, and that magnesium increases away from the organic layer (Fig. 2). Furthermore, the dolomite is not present in great quantities, and is not ordered. The conclusion that organic material has formed a site for dolomite precipitation is, however, inescapable.

Although these experimental and field data seem to bear out the convictions of Gebelein and Hoffman[1] that dolomite formation is related to organic material we can suggest a different chemical mechanism from that suggested[1] by those authors.

Their data show that no precipitation occurred unless $(NH_4)_2CO_3$ was present. Therefore, the presence of magnesium alone on organic material is of little consequence, irrespective of the Mg–Ca ratios. During their experiment[1], no organic decomposition occurred. Magnesium was therefore not made available for precipitation by organic decomposition, but was scavenged from its organic site by the introduction of $(NH_4)_2CO_3$. Our second conclusion is therefore that the catalytic force in the crystallisation of 17–20 mol % magnesium carbonates is the increase in alkalinity. The concentrations used by Gebelein and Hoffman[1] were sufficient to increase markedly the alkalinity of the sample solutions. That this was meant to simulate organic decay is irrelevant. Organic decay did not occur. The data provide an insight into the true relationship between magnesium, alkalinity and the crystallisation of magnesium carbonates.

In most field studies the role of the Mg–Ca ratio has been stressed as critical to the formation of dolomite. Little is ever said of the CO_3 concentration. We believe that conditions for crystal precipitation depend on two parts of a solubility product. The importance of the Mg–Ca ratio is not, however, surprising when it is realised that, with only one exception, no published alkalinity data exist for dolomite precipitating environments. Lippman[5] has also warned that too much importance has been attached to studies of the Mg–Ca ratio. The solubility product of dolomite is exceeded in seawater[7–9] but dolomite does not form. Is it also coincidental that the carbonate concentration of seawater is low?

The three modern dolomitic environments which have received most treatment are Bonaire, in the Netherlands Antilles; the Coorong, in South Australia; and the Persian Gulf. Bonaire is the only major locality from which alkalinity data are available. Deffeyes *et al.*[10] report that the alkalinity is markedly higher than in seawater but, more importantly, the CO_3 concentration is greater than the HCO_3 concentration.

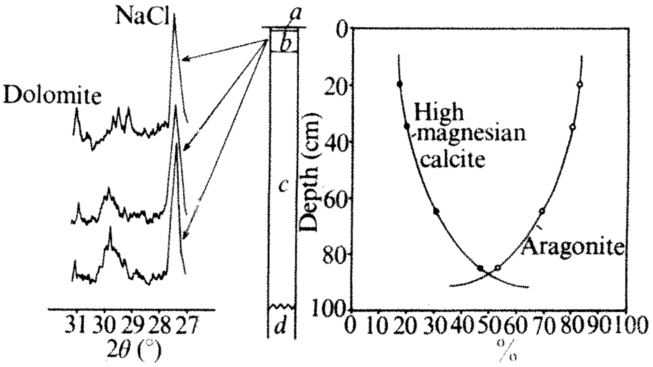


Fig. 2 Sedimentary section within the One Tree Island Pool. *a*, Algal mat; *b*, spongy pale pink to brown organic layer, *c*, precipitated high-magnesium calcite and aragonite; *d*, coral rubble. X-ray diffraction patterns of the spongy pale pink to brown organic layer show small amounts of carbonate with a dolomitic composition.

No alkalinity data have been published for the Persian Gulf localities, although it is known that salinities are high and the *p*H is low. The combination of these two factors results in the precipitation of $CaSO_4$—which leads to an increase in the solubility of previously precipitated metastable phases—and in the dissolution of these phases with a resulting increase in the alkalinity. Furthermore, a high CO_3–HCO_3 ratio is suggested by the high salinities.

Von Der Borch[11] does not record the alkalinity in the Coorong, mentioning only bicarbonate rich pore waters associated with the hydromagnesite lake. Data from Lake Fellmongery[12] show, however, alkalinity values of up to 5–6 times that of seawater; and Taylor[12] has reported Mg-calcites with 33 mol % $MgCO_3$ precipitating within the lake. Unpublished data obtained by officers of the Bureau of Mineral Resources show dolomite forming around the eastern shores of Lake Frome in South Australia: a site of carbonate precipitation that coincides with a locality at which groundwater alkalinities are high (eight times that of seawater).

We conclude, therefore, that high alkalinity is extremely important in the formation of dolomite. Organic material may play three different roles: first, living organic material, by virtue of photosynthesis, raises the *p*H and therefore raises the CO_3–HCO_3 ratio; second, living organic material can accumulate large quantities of magnesium ion[1–3]; and third, decaying organic material releases methane which may be used to reduce gypsum, with the concomitant production of CO_2. Similarly bacterial decay of organic material also produces CO_2. In both cases, the production of CO_2 will lead to an eventual increase in alkalinity. Various types of organic material, particularly some molecular fractions of humic acids inhibit the precipitation of aragonite. This inhibition may provide sufficient time to nullify the kinetic barriers of dolomite formation.

We thank D. Barnes for the X-ray determinations. K. Lundgren and B. Radke helped in the One Tree Reef programming, and facilities on the reef were provided by the Australian Museum.

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Thermal stability of silicon carbide fibres

THE potential of high strength silicon carbide fibres for use in composite materials[1] depends on their thermal stability; specifically on the ability to retain their strength after exposure to the temperatures of composite fabrication and subsequent service. Aveston[2] has reported a marked reduction in the strength of such fibres after they have been exposed to high temperatures. This has not been observed in single crystal and bulk polycrystalline silicon carbide which exhibits no degradation of mechanical strength with temperature[3,4]. We have studied the influence of high temperature on the tensile fracture strengths of pyrolytically deposited silicon carbide fibres from several sources. We found that their strengths after exposure were considerably higher than may have been expected from the previously published data.

The four pyrolytically deposited silicon carbide fibres which we examined are listed in Table 1 (fibres A–D). The silicon carbide densities of all of the fibres were less than the theoretical values and the fibre coatings had β silicon carbide structures with varying amounts of a disordered silicon carbide phase. The fibres were heated in air or high purity argon at various temperatures above 1,000 °C. Fracture strengths were measured at room temperature in tension using a gauge length of 25.4 mm. Because the fibres shattered on failure when they were tested in air, all of the tests were performed in an organic liquid (polypropylene glycol 2025) which was sufficiently viscous to prevent shattering but had no significant effect on the fibre fracture strengths. For example, the mean strength of fibre A from six tensile tests in air was $970+100$ MN m^{-2} compared with a mean strength of $970+90$ MN m^{-2} for the same number of tests conducted in polypropylene glycol. This procedure permitted the identification and elimination of results from specimens which failed outside the gauge length because of poor alignment, and preserved primary fracture faces for subsequent examination by scanning electron microscopy (SEM).

Table 1 compares the initial strengths of the fibres with their strengths after heating for 48 h at 1,200 °C in air and argon, respectively. The data can also be compared with those obtained by Aveston[2], although our fibres were present in the hot zone of the furnace throughout the temperature cycle and, consequently, spent about 55 h above 1,000 °C. The mean strengths of our tungsten substrate fibres (that is, fibres A, B and C) after exposure for 48 h at 1,200 °C were found to be between 2.4 and 3.7 times the mean strength of the fibre subjected to similar treatment by Aveston[2]; the carbon substrate fibre (D) is up to six times stronger, with a mean strength of 1,480 MN m^{-2} after treatment in air (Table 1).

SEM studies of the fracture faces of fibres A, B and C detected the formation of voids at the original tungsten–silicon carbide interface (Fig. 1*a*) after treatment at 1,200 °C. The voids could well represent the flaws in the substrate region discussed by Aveston[2], but we observed that the tungsten in our fibres had reacted to form tungsten silicide phases as well as tungsten carbide. In most cases, however, the flaw controlling the strength was identified as a surface flaw of the type shown

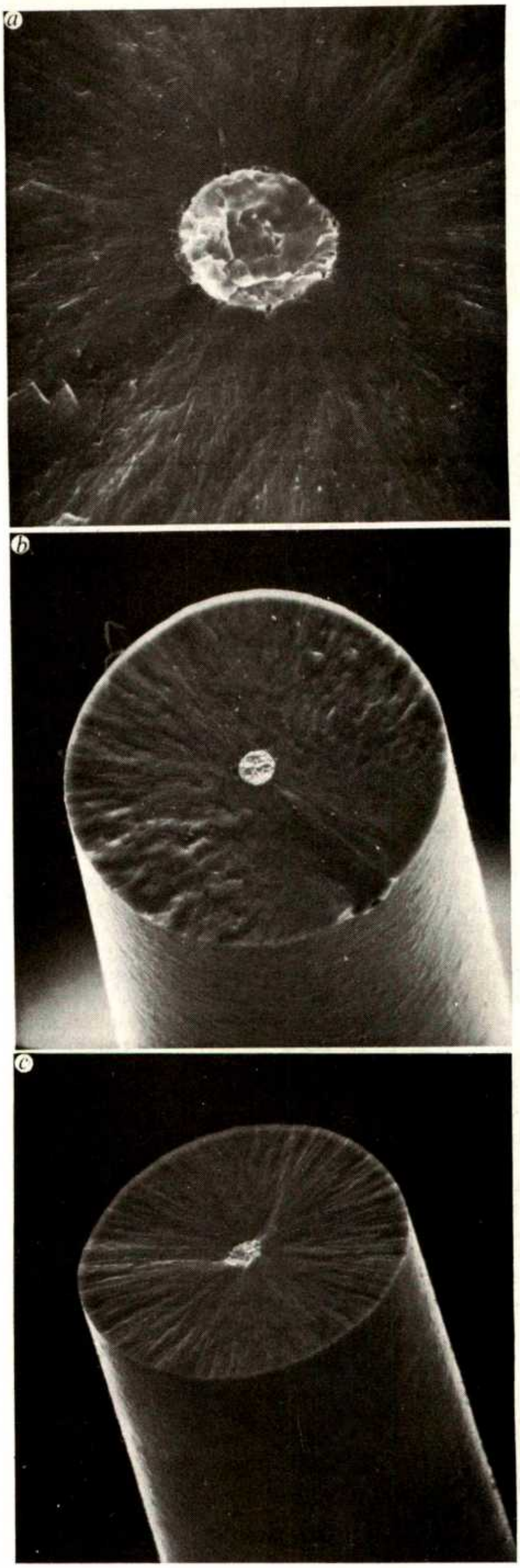

Fig. 1 Fracture surfaces of silicon carbide fibre B heated for 48 h at 1,200 °C. *a*, Voids at tungsten–silicon carbide interface (× 1,680); *b*, surface initiated failure (× 420); *c*, failure probably initiated at tungsten–silicon carbide interface by flaws of type shown in *a* (× 375).

Table 1 Room temperature fracture strengths of fibres before and after heating in air and argon

Fibre*		Initially Mean strength (MN m^{-2})	No. tested	After 48 h at 1,200 °C in air Mean strength (MN m^{-2})	No. tested	After 48 h at 1,200 °C in argon Mean strength (MN m^{-2})	No. tested
A	(this work)	970±90	6	700± 70	6	600±30	5
B	(this work)	2,990±530	12	660± 40	8	730±20	5
C	(this work)	4,000±140	6	920±130	7	740±90	5
D	(this work)	3,990±380	7	1,480±110	6	810±90	5
E	(Aveston[2])	2,060		240± 30		250±30	

*Fibre A—100 μm diameter with 25 μm tungsten wire substrate; fibre B—100 μm diameter with 12.5 μm tungsten wire substrate; fibre C—150 μm diameter with 12.5 μm tungsten wire substrate; fibre D—100 μm diameter with 33 μm carbon fibre substrate; fibre E—100 μm diameter with 12 μm tungsten wire substrate.

in Fig. 1*b*. Over 80% of the type A and B fibres treated in air and argon and 50% of the type C fibres heated in argon exhibited fractures initiated at surface flaws. In the case of fibre C heated in air, less than 20% of the failures originated at surface flaws. When surface flaws were not identified as the origin of failure, the critical flaw was difficult to define (Fig. 1*c*) but the evidence suggests that flaws at the substrate of the type illustrated in Fig. 1*a* are a likely source of failure.

In the case of the carbon substrate fibre D all failures were attributed to surface flaws after heating in both air and argon, though surface attack was less severe when the fibres were heated in air. As expected, no evidence of reaction between the carbon substrate and the silicon carbide was observed, suggesting that extremely good strength retention might be achieved if surface attack could be avoided.

The important conclusion from our data is that tungsten substrate, silicon carbide fibres available at present retain a useful proportion of their strength after exposure for 48 h at 1,200 °C. Thus, their potential as a high temperature reinforcement in metal or ceramic composites is considerably greater than has been indicated in the past[2].

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Received December 16, 1974, accepted April 28, 1975.

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^{14}C carbonate dating and the age of post-Talayotic lime burials in Mallorca

THE study of the cultures of Mallorca in the Balearic Islands, normally recognises pre-Talayotic, Talayotic and post-Talayotic phases. The pre-Talayotic phase covers the interval preceding the adoption of megalithic building techniques. The period of construction of the Talayot towers lasted from about 1500 to 500 BC and was followed by a post-Talayotic phase during which the Talayotic culture underwent a rapid degeneration[1,2]. In the last centuries of the Talayotic period, Phoenician and Greek influences are evident. Ibiza, about 100 km by sea from Mallorca, was settled by the Phoenicians in 654–653 BC. The Balearics were also involved in wars between Rome and Carthage, between the fifth and second century BC, an involvement which culminated in the conquest of Mallorca in 123 BC by Caecilius Metellus.

Two different definitions of the post-Talayotic phase exist. Garcia[2] associates the phase with the period following the Roman conquest but Rossello Bordoy[1] gives it as the period during which the true Talayotic culture gave way to a decline that lasted until the 'Romanisation' of the island. We accept the latter definition of post-Talayotic.

During the post-Talayotic, burial customs changed drastically. Normal earth interments were replaced by lime burials, and the use of iron objects among the grave goods became more common[3]. Lime interments are not found elsewhere in the Mediterranean, and they constitute a strange Mallorcan custom. The origin of the phenomenon is not well understood. Our ^{14}C dates indicate that the custom originated in the fifth to sixth century BC. Contact with the Phoenicians had already been made by that time, and may have led to the introduction of general lime making techniques. At the beginning of the early Iron Age the Phoenicians in the Canaanite east developed a new method of lining water storage cisterns with impermeable lime plaster for the long term storage of rainwater. Later, a true cement was developed, and many cisterns with such linings exist from the later years of Punic Carthage[4]. The Phoenicians, however, did not use lime burials. Even though the Mallorcan inhabitants may have been familiar with lime making techniques through Phoenician contacts, it is not clear why the technique was adopted for interments.

We examined lime carbonates and incorporated charcoals to determine the feasibility of lime dating for this environment., The majority of the samples we collected, in 1969 and 1973 came from the Son Matge burial site, Mallorca (2°25′E′ 39°35′N).

Four charcoal samples from the Talayotic interment phase, taken from directly underneath the lime burial strata, were collected from several excavated sections. The laboratory numbers (QL = Quaternary Isotope Laboratory) and ^{14}C ages (yr b.p.) of these samples are QL-10, 2,480±70; QL-11, 2,700 ±80; QL-26, 2,520±80; and QL-27, 2,640±50 respectively. This places the transition from 'regular' burial techniques to lime burial techniques at approximately 2,500 yr b.p. The ^{14}C age is equivalent in calendar years to 510–660 BC, according to the MASCA calibration tables[5].

The lime strata in the Son Matge burial grounds have thicknesses of up to 1 m in the central area. A multitude of bone remnants is found in the lime carbonate, but no collagen is left for ^{14}C dating, because of the high temperatures achieved during lime slaking. Isolated charcoal samples were found in two sections at depths of 75 and 90 cm in the lime layer, close to the contact with the Talayotic burial phase. The ages of these samples (QL-6, 2,520±80 yr b.p. and QL-4, 2,540±110 yr b.p.) confirm that the transition of burial types probably occurred about 2,500 yr b.p.

Charcoal from a shallower section (at 45 cm depth) gave an age of 2,080±90 yr b.p. (QL-8). To this can be added two bone charcoal dates from lime related burials at Son Puig and Muertos Gallard[2,3] with ages of 2,180±100 and 2,230±80 yr b.p. (Y-1791 and Y-1859, respectively). Evidently, lime burials lasted at least until about 2,100 yr b.p., which is equivalent to a calendar age of 70–110 BC (ref. 5). This post-Talayotic episode could not have lasted much longer, as Roman artefacts occur directly above the lime layer. The curious phenomenon of lime interment was probably practised for about five centuries, from about 600 to 100 BC.

Two pairs of lime carbonate–charcoal samples (QL-8/9 and QL-6/7) were available for a test of the reliability of ^{14}C dates from the carbonate. The ages of these pairs agree with each

Table 1 Results of Son Matge analyses

Sample no. and location	Depth below surface of burial layer (cm)	$\delta^{13}C$ (‰)*	^{14}C age (yr b.p.), (corrected for isotope fractionation)
Post-Talayotic lime burial strata			
QL-8 charcoal, section 36	45	−23.0	2,080±90
QL-9 carbonate, section 36	30	−16.8	2,200±100
QL-6 charcoal, section 33	75	−23.4	2,520±80
QL-7 carbonate, section 33	75	−19.9	2,730±100
QL-4 charcoal, section 37	90	−22.5	2,540±110
Y-2669 carbonate, exploratory trench	40	−18.3	2,400±80
Y-2672 carbonate, exploratory trench	80	−13.6	2,240±80
QL-22 carbonate, section 44	20	−11.7	2,260±60
Samples probably associated with Talayotic fire levels			
QL–23 carbonate, section 33	75	−17.7	4,020±50
QL–5 carbonate section 37	about 90	−19.0 −19.9 −20.9	3,970±100 3,420±100 3,350±60
From Talayotic fire level			
QL–24 'habitational' lime-carbonate	125	−21.4	3,670±70

$$* \ \delta^{13}C = \frac{(^{13}C/^{12}C_{\text{sample}} - {}^{13}C/^{12}C_{\text{standard}})}{^{13}C/^{12}C_{\text{standard}}} \times 1{,}000$$

other within the limits of statistical error (Table 1). The ages of three other carbonate samples (Y-2669, Y-2672 and QL-22) also fall in the period of lime interment between 2,500 and 2,100 yr b.p. But two of the carbonate samples (QL-5 and QL-23) are definitely too old and deviate by 900 and 1,500 yr, respectively, from the oldest lime interment ages.

Both carbonate samples with the older ages are found close to the contact zone between the lime burial deposit and the Talayotic fire levels. The lime burials are not always at the same depth, and Talayotic fire levels exist within centimetres of the burial zone. It is possible that the older carbonate was formed after local limestone bedrock was decomposed by overlying Talayotic fires. Sample QL-24 is a clear example of such 'habitational' lime carbonate; its age of 3,670±70 yr b.p. is in excellent agreement with the age of the charcoal from associated fires. In our opinion, the two older carbonate samples are associated with Talayotic fire levels, and are not part of the lime burial deposit.

The $\delta^{13}C$ values listed in Table 1 represent the deviation (‰) of the sample $^{13}C/^{12}C$ ratio from the PDB standard. The $\delta^{13}C$ values of the carbonate samples of 'normal' ^{14}C age average −16.1‰. The lime at Son Matge has probably been made from the limestone cave wall which has a $\delta^{13}C$ value of −1.4‰. If the older ages of the two carbonates already mentioned had been caused by incomplete outgassing of the limestone during lime fabrication, the expected $\delta^{13}C$ values would be higher. Age differences of 900–1,500 yr could be caused by the addition of up to 20% bedrock limestone, resulting in a maximum increase in the $\delta^{13}C$ ratio of 3‰. But the $\delta^{13}C$ values of the 'anomalous' samples average −19.4‰; 3.3‰ below the −16.1‰ found for the carbonate samples with the proper ^{14}C age. This means that incomplete outgassing is unlikely to be the cause of the older carbonate ages.

This analysis indicates that ^{14}C dating of carbonate associated with lime burials, yields reliable ages. Unfortunately the range of $\delta^{13}C$ values, from −12 to −21‰, is largely a result of kinetic effects. Thus, anomalous ages cannot always be detected using ^{13}C ratios. Interment lime dating, on average, yields much better results than mortar dating, but a criterion that always distinguishes normal material from anomalous material is not yet available.

The accurate ^{14}C dating of mortar depends on the extent of contamination by ancient carbon resulting from the use of calcareous sands and/or incompletely kilned limestone[6-8]. As these factors depend on geographical location, a few Mallorcan samples were measured. Stucco from the Pollentia Roman Republican building at Alcudia, dating from about 30 BC, has a very anomalous age of 8,000 yr b.p. (Y-2668). On the other hand, stucco from the Santa Catelina de Sena excavation, dated (through its association with Moorish pottery) at about 1,000–1,200 AD, has a ^{14}C age of 950±100 yr b.p. (Y-2671). Evidently, the lime making techniques of these two cultures were quite different.

We also investigated the ^{14}C age of lime carbonate at the Son Oms site, formed after the local limestone bedrock was decomposed by overlying fires. There, a completely anomalous age of 20,750 yr was obtained (QL-19), but in that instance the $\delta^{13}C$ ratio of −5‰ clearly showed that incomplete degassing of the original limestone was the cause of the discrepancy.

This work was supported by the US National Science Foundation.

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Mechanical state of elastin

WE describe experiments in which the mechanical state of elastin was determined in conditions comparable to those used by Weis-Fogh and Anderson[1,2]. In their mechanocalorimetric experiments a strain was imposed on a specimen immersed in diluent in a calorimeter, maintained for several hundred seconds, and then released. Measurements of heat release led them to reject the classical theory of rubber elasticity[3-6] which, for solvated elastin, they replaced by a new model, the liquid-drop elastomer. There is, however, good evidence that in neglecting the differential heat of solution Weis-Fogh and Anderson neglected a dominant source of heat release[7,8]. Our purpose is to examine the alternative assumption, that elastin conforms to the classical theory of rubber elasticity in the experimental conditions they studied.

A small elastin ring (sliced from pig aorta and purified according to the method of Gotte *et al.*[9]) was mounted in an inverted torsion pendulum[10], immersed in solvent and held in a vertical position hooked over supporting metal wires. The ring was elongated by a very small tensile load so that the specimen formed two parallel lamellae, slightly displaced from one another. Let the dimensions of each lamella at temperatures T_0 and T be a_0, b_0, c_0 and a, b, c. Let the specimen be isotropic so that if $a = \gamma_T a_0$ then $b = \gamma_T b_0$ and $c = \gamma_T c_0$ in which γ_T

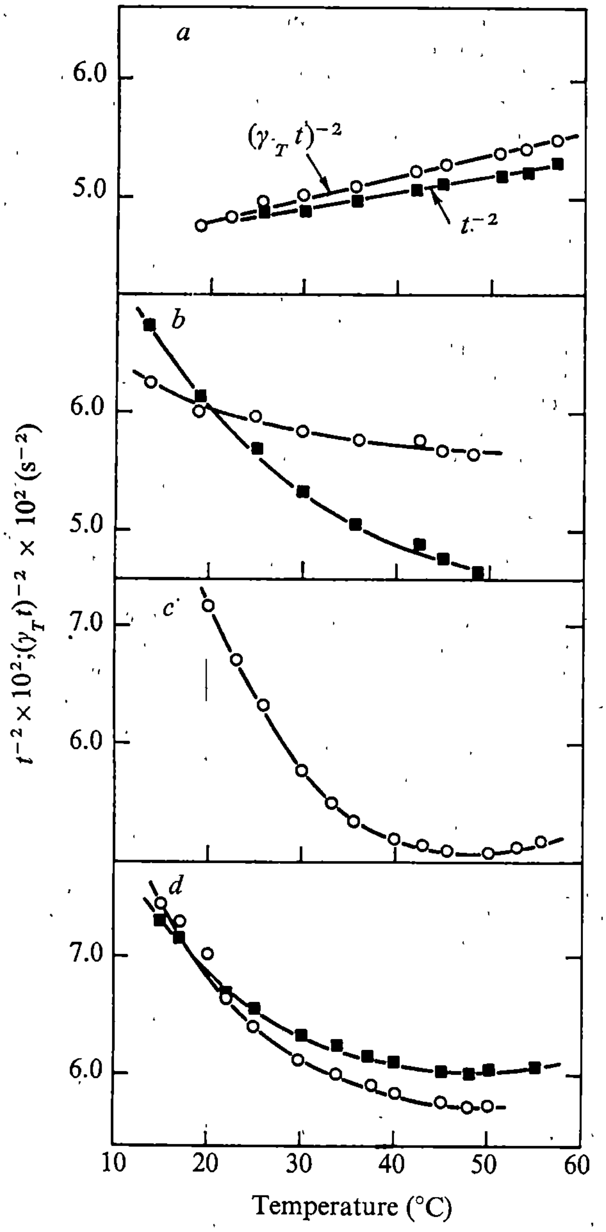


Fig. 1 Temperature dependence of the inverse square of the period of oscillation of the torsion pendulum corrected (○) and uncorrected (■) for change in specimen dimensions as a result of swelling in water–ethanol solutions and formamide. The theory of rubber elasticity predicts the plot of $(\gamma_T t)^{-2}$ as a function of T to be linear and of positive slope (equations (4) and (5)). *a*, Formamide; *b*, water; *c*, water–20% ethanol; *d*, water–50% ethanol.

is a parameter which depends only on temperature. It follows that if the moment of inertia of the pendulum is constant the ratio of the shear moduli at T and T_0 is[10],

$$G_T/G_{T_0} = (1/\gamma_T{}^3)\,(t^{-2}/t_0{}^{-2}) \qquad (1)$$

in which t and t_0 are the observed periods of oscillation of the pendulum at T and T_0. It was not possible to observe the logarithmic decrement of the specimen because of the viscous drag of the solvent in which the specimen was immersed. The period t (~4 s) was determined at different temperatures with a stop watch. The temperature dependence of solvent uptake was obtained by weighing and γ_T was computed assuming the density of the absorbed diluent to be equal to the liquid density.

According to the prediction of the theory of rubber elasticity[11],

$$G_T/G_{T_0} = T/T_0(V_{T_0}/V_T)^{1/3}\langle r^2\rangle_0{}^{T_0}/\langle r^2\rangle_0{}^{T} \qquad (2)$$

in which V is the specimen volume in equilibrium with the diluent and $\langle r^2\rangle_0$ is the mean square end-to-end distance of the network chains unrestricted by cross links (at temperatures T and T_0 as indicated). It follows that

$$\mathrm{d}\ln G/\mathrm{d}T = 1/T-(1/3)\,\beta_V-\beta_{\langle r^2\rangle_0} \qquad (3)$$

in which β is the temperature coefficient of volume and of $\langle r^2\rangle_0$ according to subscript. For water at 20 °C and taking account of the errors involved in measuring β_V and $\beta_{\langle r^2\rangle_0}$ we may neglect $\beta_{\langle r^2\rangle_0}$ ($= 0.5\times10^{-3}$, ref. 6) in comparison with $1/T$ (3.41×10^{-3}) and $\beta_V/3$ ($= -4.5\times10^{-3}$, ref. 6). Since the value of $\beta_{\langle r^2\rangle_0}$ for water seems unusually large (compared with ethylene glycol and dimethyl sulphoxide[6]) and in the absence of evidence to the contrary (see also ref. 7) we take $\beta_{\langle r^2\rangle_0}$ to be negligible for water–ethanol solutions and for formamide. Therefore rewriting equation (2) with $\langle r^2\rangle_0{}^T/\langle r^2\rangle_0{}^{T_0} = 1$ and $\gamma_T = (V_T/V_{T0})^{1/3}$

$$G_T\gamma_T = AT \qquad (4)$$

in which the constant $A = (G_{T0}/T_0)$. From equation (1), the observed value of $G_T\gamma_T$ is

$$G_T\gamma_T = B(\gamma_T t)^{-2} \qquad (5)$$

in which B is a constant equal to $G_{T_0}t_0{}^2$. So a specimen conforming to this rubber elastic model will exhibit a linear dependence between $(\gamma_T t)^{-2}$ and T. If the transition from the glassy to the rubbery state is incomplete and the specimen significantly viscoelastic then a plot of $(\gamma_T t)^{-2}$ against T will not be linear and will be of negative slope[10,12,13].

It is not the purpose of these experiments to determine precisely the relative magnitude of f_e and f_s, the energy and entropy components of the mechanical force. The torsion pendulum is not well suited for this task. The purpose is much simpler; briefly stated, it is to determine whether or not the temperature dependence of $G_T\gamma_T$ is negative or positive.

The temperature dependence of t^{-2} and $(\gamma_T t)^{-2}$ is shown in Fig. 1 for elastin immersed in formamide, pure water and water–ethanol solutions containing 20% and 50% ethanol (v/v). The elastin–formamide system shows a linear dependence between $(\gamma_T t)^{-2}$ and T with a positive slope in accordance with the theory of rubber elasticity. The behaviour of elastin in the other solvents is typical of an amorphous polymer at temperatures in the viscoelastic transition zone; the plot of $(\gamma_T t)^{-2}$ as a function of T is a curve of negative slope[10,12,13]. This important finding was checked using step-function experiments at greater mechanical strains.

An elastin ring was looped over a pair of Invar rollers (held in an Invar jig) and mounted in an Instron testing machine. The elastin ring was immersed in diluent at constant temperature. At the start of a stress relaxation experiment ($t = 0$) the roller separation was changed rapidly (within 1 s) from one predetermined position to another and then maintained at this second position. The roller displacement was calculated to yield a strain in the specimen close to 30%. The relaxation of the force was observed out to $t = 190$ s, the first measurement being taken at $t = 2$ s. This was repeated at temperatures in the range 0–60 °C, the specimen being allowed to relax between experiments. At each temperature the machine moved the rollers automatically through the identical displacement.

The temperature dependence of the force $f(t)$ is shown in Fig. 2 for elastin immersed in 20–80 ethanol–water solution. The interpretation for this system is particularly straightforward since γ_T is close to unity in the temperature region

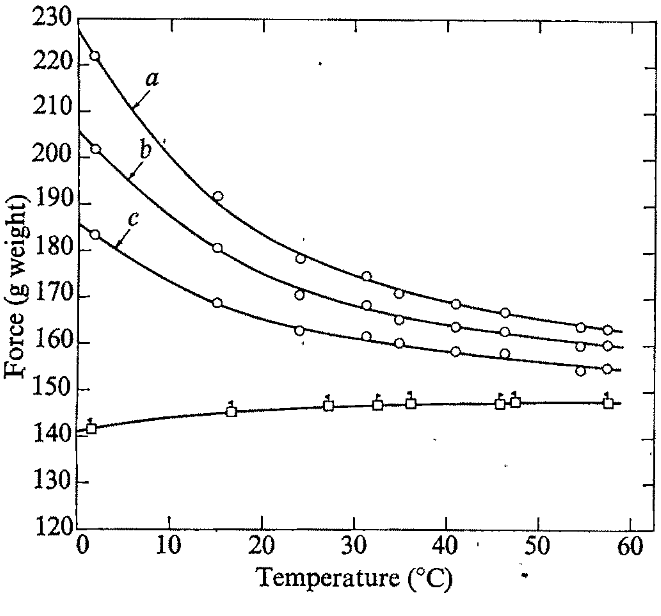
0–60 °C. The observed behaviour is in complete accord with the torsion pendulum experiment. The temperature dependence of $f(t)$ (negative and curved) and the time dependence (greatest at lowest temperature) is typical of an amorphous polymer in the viscoelastic transition zone.

The relationship of the determined values of $f(t)$ to force–temperature observations obtained in a conventional thermoelastic experiment was made clear in the final experiment. After the final determination of $f(t)$ at 58.4 °C the specimen was maintained at that temperature under constant length for 120 min. The value of the force at this time, designated f_R, is shown in Fig. 2. The temperature was then lowered and measurements of f_R taken at five temperatures between 58.4 and 1.6 °C (Fig. 2). The reversibility of the f_R–T curve was checked by taking two further f_R points at 32.6 and 45.8 °C (Fig. 2). It will be seen that f_R is of positive slope at low temperatures and essentially zero slope above 50 °C.

The usual thermoelastic procedure for studying elastomers is to deform at the highest temperature for some time (for example, overnight[6]) and then to observe the temperature dependence of force at constant length. The resulting force temperature plot is analogous to the f_R–T plot of Fig. 2. But this experiment does not measure the mechanical state in the conditions described by Weis-Fogh and Anderson. The essential difference is one of time: short time (10–1,000 s) in their experiment, and pseudo infinite time in the determination of f_R. For the elastin–formamide system this difference in time scale is not significant: our observations show that the $f(t)$ and f_R plots (as functions of T) are of positive slope and essentially parallel. It follows that for this highly swollen system the theory of rubber elasticity is adequate. But for the less swollen elastin–water and elastin–water/ethanol systems the time dependent force $f(t)$ will depend on other parameters in addition to f_R, notably the distribution of relaxation times for the relaxation process.

It follows that elastin in the vasculature operates under conditions of time, temperature and solvent uptake which place it in the viscoelastic transition zone. The equivalent time in step function experiments may be approximated, $t = (2\pi\upsilon)^{-1}$ in which υ is the oscillation frequency: for $\upsilon = 1$ Hz $t = 0.16$ s. Stress relaxation experiments at times down to 2 s on the elastin–water system support the observation shown in Fig. 1 that at body temperature and under the oscillatory conditions of the vasculature elastin will be significantly viscoelastic. The most significant physiological implications of this are mechanical damping and the response of the solid to the hydrostatic component of the stress tensor. As emphasised by Hoeve and Flory[7] the volume of the swollen specimen increases with length; $(\partial V/\partial L)_{pTe_q} > 0$ in which eq denotes the specimen is in equilibrium with a reservoir of diluent. When the stress is applied the specimen absorbs liquid which is desorbed when it is released. Studies of non-diluted polymers show this volume change to increase when the specimen is cooled from the rubber elastic into the viscoelastic transition zone[14,15]. If this is true for diluted elastin then a pulsating stress in an element of elastin will generate a molecular flux along a concentration gradient which may well be considerably larger than the flux due to normal diffusion processes. The efficiency of this stress assisted diffusion will be facilitated by a laminated texture, as in the vasculature.

Fig. 2 Temperature dependence of force at constant length for elastin in 20–80 ethanol–water solution; ○, $f(t)$ for values of t equal to 2 s (a), 10 s (b), 190 s (c); □, f_R. The flag on the f_R points indicates direction of temperature change.



We note finally the deviation of the f_R–T data from those near in form to proportionality between equilibrium force and absolute temperature, which is predicted[5] for a system in which: (i) the internal energy contribution to total retractive force is small $(f_e/f \ll 1)$; (ii) disproportionation of solvent within the polymer phase is negligible; (iii) equilibrium swelling is independent of temperature ($\gamma_T = 1$). Our work indicates that although γ_T varies almost imperceptibly with temperature in the 20–80 ethanol–water solution, the temperature dependence has a magnitude large enough to modify the slope of the curve significantly. Furthermore, the importance of (ii) as a contributory factor remains in dispute. We do not find any discrepancy between the form of the f_R–T curve obtained and the claim of near ideal elasticity for swollen elastin in the equilibrium state $(f_e/f \ll 1)$. A more precise examination of the implications of experimental data obtained on elastin will require a realistic consideration of the small anisotropy known to be present.

We thank Dr S. M. Partridge for discussions.

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Received January 30; accepted April 28, 1975.

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Cellulase activity and niche separation in freshwater gastropods

ALTHOUGH there is considerable morphological similarity between different species of freshwater snail in terms of general body form and radular construction[1], differences in diet occur[2,3]. Furthermore, preferred food materials are invariably digested more efficiently than less preferred materials[4] (Fig. 1). The implications are that differences in diet and resultant niche separations are dependent on physiological, rather than morphological, divergence and that the former is probably related to the quality and quantity of enzyme secretion. Thus it may be

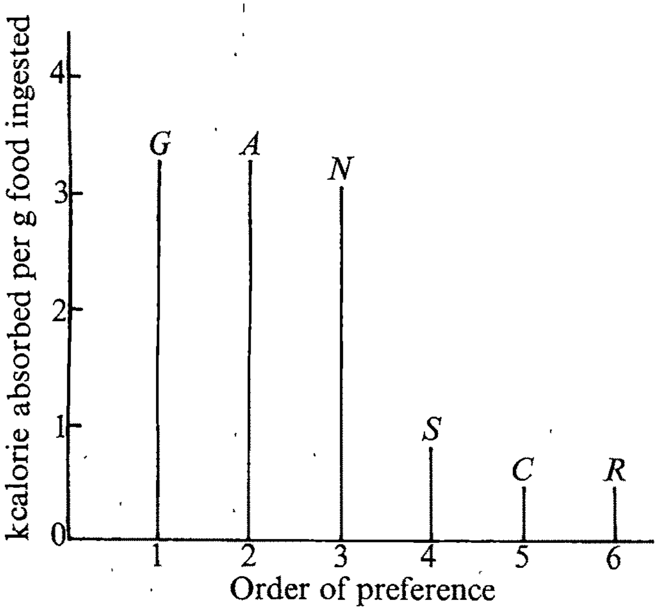


Fig. 1 Nutritive value of food, in terms of potential energy absorbed across the gut wall per weight of food ingested (derived from ref. 4), against the preference order of food materials to *Ancylus fluviatilis*. The latter were obtained from independent laboratory and field observations[13]. Intervals between the preference values are not equal and as yet have not been quantified. Similar results have been obtained for *Planorbis contortus* and *Lymnaea pereger* and suggest that snails prefer those food materials which provide most potential energy per mouthful. This dependency may not be linear. *G*, *Gomphonema*, *A*, *Achnanthes*; *N*, *Navicula*; *S*, *Scenedesmus*; *C*, *Cladophora*, *R*, *Rivularia*.

possible to reconcile the conflict first noted by Boycott[5] between the general observation that most species of freshwater gastropod can and do coexist[5,6] and the so-called "competitive exclusion principle". To test this hypothesis we have examined cellulase activity in 14 species of snail and have linked our results to known dietary differences and to direct observations on assimilation efficiencies. We have found a strong correlation between enzyme activity and assimilation efficiency, and that dietary preferences can be ascribed to physiological differences of this kind.

Snails were obtained from Malham Tarn (Yorkshire, UK). Enzymes were extracted from starved snails[7] and were used immediately. Activity measurements were made by estimating the amount of reducing sugar liberated from type II cellulose[7]. Enzyme extract (4 ml) was added to 50 mg of substrate in 1 ml of 0.1 M phosphate buffer (*p*H 5.5) and incubation was for 6 h at 4 °C. These conditions are optimum for the activity of molluscan cellulases[7]. The reaction was stopped by adding 1 ml of 0.1 N NaOH. Blank incubations were made using the enzyme extract without cellulose and cellulose without enzyme. Corrections were subsequently applied to the experimental replicates for the small amount of reducing sugar initially present in each. Protein concentrations of the original extracts were estimated from separate subsamples[8].

The efficiency with which snails digest green algae was used as an index of their ability to digest and assimilate cellulose. *Scenedesmus* sp. was used as food supply because it has comparatively thick cellulose walls which increase its resistance to herbivorous grazing[9]. Assimilation efficiencies were measured using a 'double isotope' technique involving ^{14}C and ^{51}Cr (ref. 10). All snails were starved for 3 d before use and determinations were carried out at 10 °C. It was assumed that each species absorbs about 10% ^{51}Cr as in *Planorbis contortus* L. and *Ancylus fluviatilis* Müll.[10].

The results of both cellulase activity and assimilation efficiency are given in Table 1. Previous work on cellulase activity in *Lymnaea stagnalis*[11] gave values of 272 and 263 units (as defined in Table 1) for the crop-gizzard apparatus and hepatopancreas respectively. The latter are extremely similar to our results. Throughout we have ignored questions concerning the source of cellulases in molluscs[11,12] (that is, from bacterial or snail tissue) because from a dietary point of view, all that is required is that the enzymes are present.

Gut extracts from different snail species show differences in cellulase activity (Table 1) and these reflect an underlying physiological variability. There is also a positive correlation between cellulase activity and the ability of a particular species to digest *Scenedesmus*. This is illustrated in Fig. 2, but no line is drawn through the points because the theoretical relationship between enzyme activity and digestive efficiency is not known. A simple linear relationship is, however, suggested and a cause–effect association is implied.

The results presented in Table 1 and Fig. 2 conform to the previously reported findings on dietary preferences and to the fact that snails tend to prefer foodstuffs which are easiest to digest (see Fig. 1). Thus *L. pereger obtusa* seeks out, prefers and is able to digest, green algae with thick cellulose walls[2]. It has been suggested that this emphasis on green algae may apply to the lymnaeids in general[2] and Table 1 shows that these snails do have a high cellulase activity. *A. fluviatilis*, however, has a low cellulase activity and tends to prefer epilithic diatoms without a cellulose wall but which have a tough, protective siliceous shell[13]. The gizzard musculature is better developed

Table 1 Cellulase activity and assimilation efficiency (with respect to *Scenedesmus* spp.) in a variety of freshwater gastropods

Species	Whole organism‡	Cellulase activity* Crop–gizzard‡	Hepatopancreas‡	Assimilation efficiency† (%)§
Pulmonata				
Lymnaea stagnalis L.	—	275	270	76.0 (±6.8)
Lymnaea pereger L.	—	246	210	71.5 (±8.6)
Lymnaea truncatula Müll.	256	—	—	70.1 (±5.1)
Lymnaea palustris Müll.	—	229	221	62.4 (±6.1)
Physa fontinalis L.	—	104	96	39.1 (±4.3)
Ancylus fluviatilis Müll.	28	—	—	13.9 (±3.8)
Planorbis planorbis L.	—	151	182	46.9 (±5.1)
‖*Planorbis corneus* L.	—	—	243	—
Planorbis albus Müll.	40	—	—	26.0 (±4.8)
Planorbis contortus L.	32	—	—	7.7 (±2.1)
Prosobranchia				
Bithynia tentaculata L.	—	281	284	89.0 (±9.2)
Potamopyrgus jenkinsi Smith	269	—	—	78.0 (±7.3)
Valvata cristata Müll.	340	—	—	92.4 (±10.0)
Valvata piscinalis Müll.	329	—	—	94.7 (±7.8)

*Cellulase activity $= \dfrac{\mu\text{g reducing sugar per ml incubation mixture}}{\text{mg protein per ml extract}}$

†In terms of carbon[10].

‡Average of 3 determinations; maximum range ±5–7 % mean.

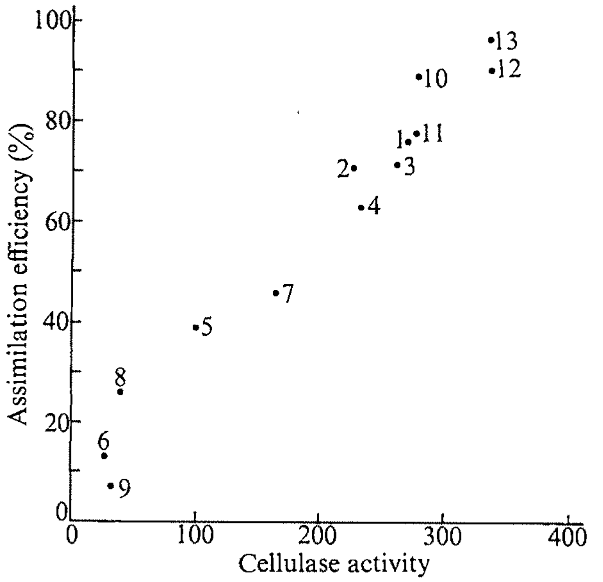
§Average of 20 determinations; confidence limits $= 2s/\sqrt{n}$ where s is standard deviation, n is 20.

‖Obtained from Stone and Morton[11].

in this species[13], so here the emphasis seems to have shifted from that of digesting the cellulose walls chemically, to that of rupturing the diatom shells mechanically. Cellulase activity in the Planorbidae is more variable. The larger species (for example, *Planorbis planorbis* and *P. corneus*) have an active cellulase and with a predominantly detrophagous habit[2] may be able to make use of the non-living, organic, detrital fraction (that is, cellulose). The smaller species like *P. contortus*, however, have a low cellulase activity and this may result in (or be the result of) a bacterial feeding mode[14]. Little is known about the dietary requirements of the freshwater prosobranchs but as suggested by the high cellulase activity, they do seem to feed on green plants and/or detritus. For example, *Bithynia tentaculata* feeds on water weeds for most of the year but feeds on detrital particles during the winter[15] and one of us (P.C.) has observed some populations of *Potamopyrgus jenkinsi* feeding exclusively on detritus and others feeding on algal epiphytes in which green unicells were dominant (unpublished).

Although the variations discussed are limited to feeding behaviour, this aspect of the niche is believed to be critical in determining the presence, absence and intensity of competition between species[16]. Differences in food requirements may also impose subtle differences on microdispersion patterns[6] which can lead to corresponding spatial separation; from this point of view it is interesting to note that Harman[17] has found a convincing relationship between substrate diversity and species diversity in freshwater snail communities. The problem of coexistence can thus be solved, in a general way, by reference to physiological variations leading to distinctions in the food, and possibly the living space requirements of different taxonomic groups. This, of course, does not completely preclude the possibility of competition and there are examples of replacement and competitive exclusion in snail communities. These, as might be expected, however, involve species with similar enzyme activities and similar dietary requirements; notably the lymnaeids and the prosobranchs. For example, *L. pereger* tends to replace both *L. truncatula* and *L. palustris*, which are typical fugitive species (unpublished), and Harman[18] has recorded competition effects between *Bythinia* and pleuroceriids. In addition, starved snails become less selective in their feeding behaviour[4] and when faced with algal monocultures in laboratory conditions, they tend to ingest more of the least well digested food materials[4]. This means that reductions in the supply of preferred food, on either a spatial or temporal scale, can lead to less discriminate feeding and may result in temporary niche overlap.

Fig. 2 The efficiency with which snails digest *Scenedesmus* spp. against the cellulase activity (defined in Table 1) of their gut extracts. Where the activity of crop-gizzard apparatus and hepatopancreas were determined separately these have been averaged. 1, *Lymnaea stagnalis;* 2, *L. pereger,* 3, *L. truncatula;* 4, *L. palustris;* 5, *Physa fontinalis;* 6, *Ancylus fluviatilis;* 7, *Planorbis planorbis;* 8, *P. albus;* 9, *P. contortus;* 10, *Bithynia tentaculata,* 11, *Potamopyrgus jenkinsi;* 12, *Valvata cristata;* 13, *V. piscinalis.*



Our results on cellulase activity support the hypothesis that in freshwater snails, niche separation depends on physiological rather than morphological differences. Snails seem to have reached an adaptive peak morphologically and subsequent niche separation seems to have occurred by physiological divergence. What is more, this may operate at an intraspecific level[19] so that differences in cellulase activity and thus dietary requirements may occur between different, but separated, populations of the same species. Physiological, as opposed to morphological, divergence also seems to be important in the Drosophilidae[20], in the Oligochaeta[21] and probably in the Nematoda. Coexistence therefore becomes possible in snails in spite of the superficial similarity of different species. Where enzyme activity is similar between species there is *prima facie* evidence for competition and this may also occur between more divergent species in conditions of food shortage.

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Received October 29, 1974; accepted April 18, 1975.

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Retinal resistance barriers and electrical lateral inhibition

EXTRACELLULAR fields around active neural tissues are usually only a small fraction of the transmembrane voltages generating them, but this is not so in the arthropod compound eye. Light-evoked fields (the electroretinogram, ERG) can range over 10 mV amplitude[1] and grow even larger near the receptor endings at the first synapse, the lamina (Fig. 1). The physical basis for such large fields is not understood although it has been suggested that there may be barriers of high resistance in the extracellular space[2,3] across which current flow may generate large voltages. Direct measurements of extracellular resistance profiles in the insect eye reported here indicate that a barrier does exist. I also show the effect such a barrier must have on the retinal currents produced by light, which is to set up a system of electrical presynaptic inhibition on certain of the receptor terminals, acting through the extracellular space. When this neural circuit is defined in detail it can be shown that it specifically selects for inhibition only those neighbouring receptors with properties dissimilar to the excited elements. Such a system might be of general interest as a mechanism of inhibitory interaction, since it can act in a graded manner, needs no neural wiring to set it up, and no genetic programme to account for the selectivity its inhibition shows. The developmental problem of how a neurone can establish highly specific connections with follower cells[4-6] and yet at the

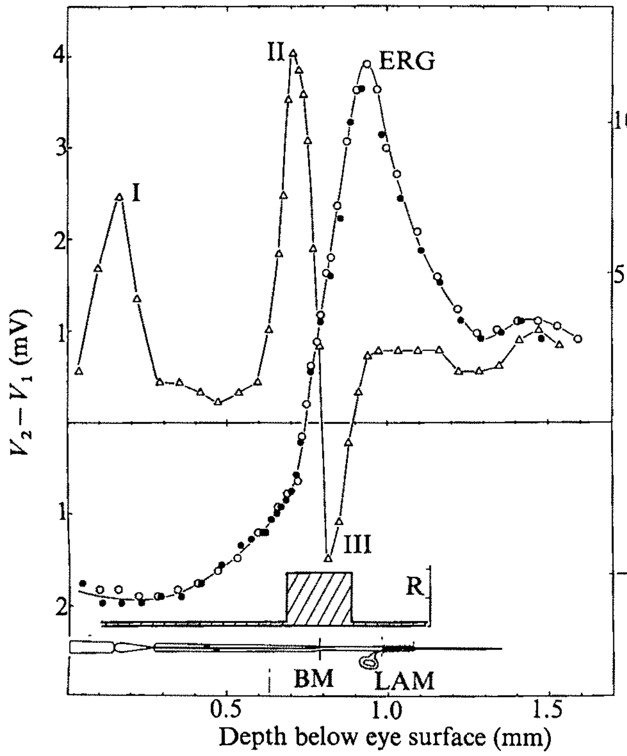

Fig. 1 Simultaneous measurements in locust eye of the depth profiles of ERG peak amplitude (○, ●) and apparent relative retinal resistance (△). The latter expresses the voltage difference produced between two electrode tips separated longitudinally by 115 μm, as 5 μA constant current pulses pass axially through the eye. There are three peaks in its profile, within the deepest of which (III) extracellular current flow is reversed, indicated here by the convention of inverting the plot. The approximate actual resistance profile that gives rise to the apparent measured curve in a model of the eye is shown below (*R*, arbitrary scale). The ERG reaches a large positive maximum near the lamina synapse (LAM) and reverses polarity close to the basement membrane (BM), but the unidirectional gradient of its depth profile down to about 1 mm depth indicates that net extracellular current flow induced by light is directed towards the periphery along most of the length of the receptors. The lower inset gives for comparison the average position of some retinal landmarks determined histologically, although the basement membrane and lamina are probably located about 100 μm more distally in this particular eye.

same time form a perhaps equally specific set of laterally-interacting connections with a quite different set of cells, is avoided with this particular mechanism.

Transretinal resistance was estimated in the outermost 2 mm of the eyes of intact locusts, *Schistocerca gregaria*, by methods similar to those for vertebrate eyes[7-9]. Isolated, bidirectional pulses of constant current, passed across the retina from a carefully positioned silver harness, produced reasonably uniform lateral fields in the retina. Assuming that current density remains constant with depth, the voltage drop in the axial direction should be proportional to the local radial extracellular resistance. The voltage drops across successive segments of extracellular space were therefore recorded between two glass micropipettes, the tips of which were separated by a fixed depth, and broken to about 20 μm to ensure extracellular recording. Each pipette also recorded the local ERG to a wide field flash. Results were taken as the electrode pair was withdrawn in steps from the eye, as the blunt tips easily snag and distort the tissue on entry.

Figure 1 is an example of the depth profiles consistently obtained, and shows three regions, I, II and III, where the local extracellular voltage drop is large. Unexpectedly, the direction of extracellular current flow in region III is reversed from that found elsewhere, and at the boundaries of III there is no net current flow at all: clearly extracellular current density is not at all uniform with depth, so that the voltage profile does not directly represent the resistance profile. To see what sort of resistance profile may reproduce this and related anomalies of the response to light and current (S.R.S., unpublished) a model of the retina has been analysed which predicts the data well with a few simple assumptions (described in detail elsewhere). Barrier II is real, and effectively seals off the extracellular space at a point part way along the receptors, starting near the basement membrane. In avoiding this obstruction, most of the applied measuring current crosses into the receptors distally, travels within them, then crosses out again in the lamina. The reversed flow noted in region III consists of this current flowing centrifugally from the lamina to haemolymph channels below the basement membrane, which form a relatively low resistance shunt around the optic lobe. Barrier I represents the progressive loss of measuring current into the

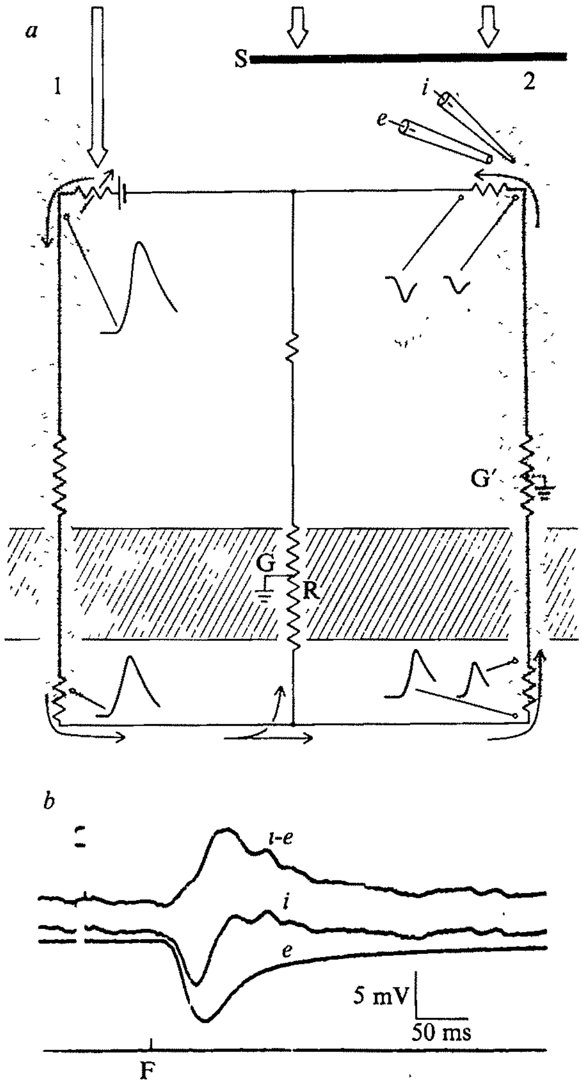

Fig. 2*a*, Extracellular inhibition of one photoreceptor by another. Photocurrents (small arrows) from excited receptor (1) complete their circuits through nearby unstimulated cells (2), because the orthodox extracellular return path is blocked by a barrier *R*. The ground reference electrode at G and the virtual ground it induces at G′ somewhere in cell 2, cause the recorded waveforms to reverse polarity as shown diagrammatically and measured in Fig. 1. The net local transmembrane voltage difference in cell 2, however, should be hyperpolarising in the axon terminals, suppressing transmitter release, but depolarising in the cell soma. *b*, This last prediction is confirmed in the cell soma of a locust photoreceptor giving a 50 mV response to direct light, but here isolated by a 100 μm diameter opaque mask (S) from a flash (F) which falls on its neighbours. The intracellular record (*i*) shows an initial negative response followed by a small positive response to scattered light. The simultaneous extracellular response (*e*) 10 μm away is even more negative, so the net transmembrane effect must be depolarising as predicted above. Electrical subtraction (*i*—*e*) confirms this.

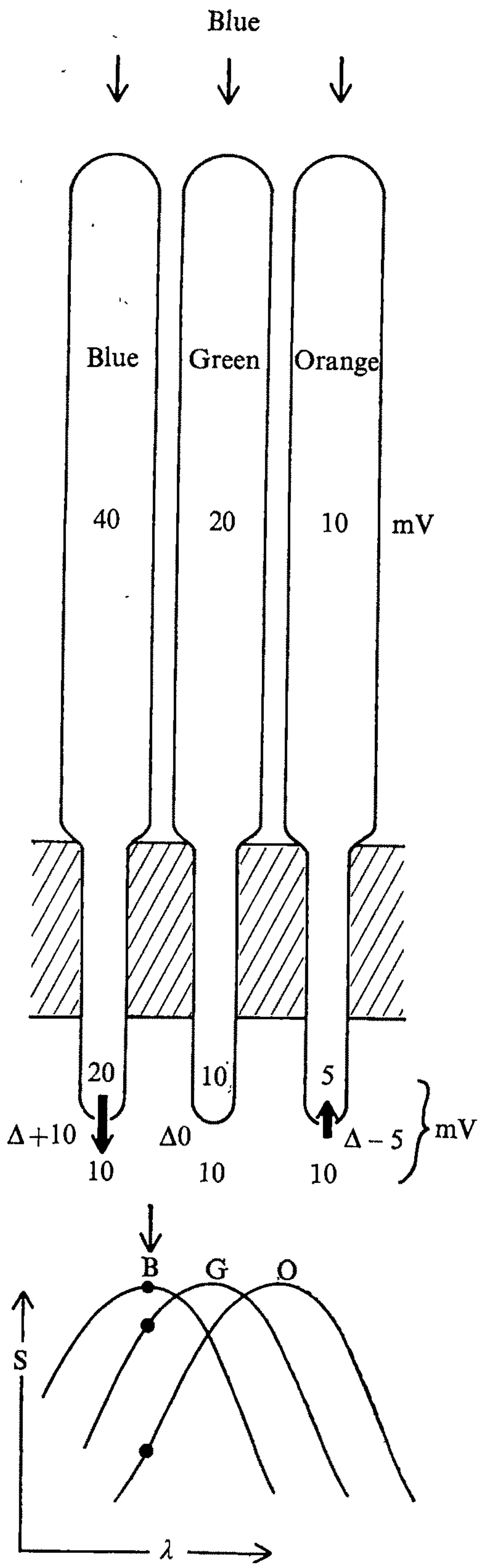

Fig. 3 Selective inter-receptor inhibition through the extracellular space, illustrated arbitrarily with three sets of receptors with differing chromatic responses exposed to blue light (S, sensitivity; λ, wavelength). Blue receptors (B) are excited strongly, thus producing a positive field around the receptor terminals. This field meets potentiometric opposition from the weaker intrinsic responses to blue light of the other two types. With the numbers chosen for illustration, this direct response is just sufficient to counter the inhibitory drive for the green (G) sensitive cells, but transmitter release will be completely suppressed from the terminals of the orange-sensitive set (O), by net inward current. Whatever the particular neuronal sets, suppression will be stronger the greater the difference in response between inhibitor and inhibited.

receptors and is not a barrier at all, and there is probably some increase in resistance with depth, reflecting the decreasing cross section of the eye down to the basement membrane. Barrier III represents the reappearance of this current, and the large voltage drop and depth profile in III indicate that barrier II must extend through region III as well, to span approximately the distance between the basement membrane and the lamina (Fig. 1, R).

The extracellular barrier affects the retinula cells by blocking off the external return path for receptor current which spreads electronically down the axons[10-12]. Just as extrinsic currents cross into the receptors to avoid the barrier, so must the returning photocurrents do the same to complete their circuit, since the barriers rectify little. The passive axons of neighbouring unilluminated receptors therefore carry most of the return current from an excited retinula cell (Fig. 2*a*). This current is directed inwards into these carrier axons, hyperpolarising them, but is directed outwards from their cell bodies back into the extracellular space of the retina, depolarising the somata. Earlier measurements suggested that this last effect may indeed occur[13], and differential recording has confirmed that the local transmembrane voltage change recorded across a carrier cell soma is depolarising as predicted, and is quite large (Fig. 2*b*). The early soma depolarisation of about 5 mV gives an estimate of the hyperpolarisation of the terminals which is necessarily a minimum, since current entry is concentrated on the terminal but its exit is distributed over the soma. The system is at least sufficiently powerful for a strongly stimulated group of receptors collectively to suppress transmitter release from their more weakly activated neighbours, to the extent of counteracting voltage changes of several millivolts in their terminals arising from perhaps 10 mV direct depolarisation of their somata.

This presynaptic, electrical inhibitory effect has several interesting consequences. First, because photocurrent will normally spread out tangentially from a single activated receptor terminal in the lamina, the extracellular field created will fall away sharply in the tangential direction from the source. The inhibitory effect of one receptor will in this case be effectively confined to a local circle of near neighbours.

Second, suppose that the eye is illuminated evenly with light, the parameters of which (wavelength for instance) are such as to stimulate one set of cells, B, more than other intermixed sets (Fig. 3). In this case tangential spread of current is minimised because of the uniform tangential voltage field[7], so that current flow becomes concentrated to produce larger radial fields. Members of set B will produce large intracellular voltages and somewhat smaller extracellular voltages in the lamina (Fig. 3). The latter act as the drive to send currents back up neighbouring axons, but if these are also type B, the drive always sees the larger opposing voltage from the strongly excited Bs: net current flux is thus always outwards (depolarising) in the terminals of the more strongly active set. For other sets, on the other hand, the net current flux could be either outwards or inwards, depending on whether the intrinsic voltage change in the particular terminals exceeds or falls below that of the local lamina drive. In general, for many classes of receptor, the dominant set(s) will suppress most severely those others with the weakest response, and have an intermediate effect on sets in between (Fig. 3). Intrinsic response differences between sets are thus accentuated by this system, but the selectivity of suppression arises not in the wiring, which is the common extracellular space, but simply in the response specificities themselves of the different neural sets, which result in differing potentiometric imbalances at the presynaptic membrane. The singular advantage of this type of selectivity that is difficult to programme with a connectivity-dependent scheme, is that it still functions properly and proportionally if differing attributes such as wavelength and polarisation-plane sensitivity are mixed up in the same neurones[14]. A possible drawback is that particular sets of cells cannot be exempted from inhibitory effects, as they could with a selective wiring scheme.

Note that when a particular stimulus activates all the receptors in one area quite strongly, the radial field which develops around the terminals will oppose transmitter release generally in that group. An automatic gain control, able to light adapt and extend the working range of the retina, is built into the first synapse[15,16].

Whether the extracellular inhibitory system described above is used to its full potentialities in the arthropod retina is not yet known. It could, for example, refine the rather imperfect polarised light response shown by crustacean photoreceptor somata[17] into a transmitter output showing an almost infinite apparent dichroic ratio, the exact result depending on the presently unknown relationship between transmembrane voltage and transmitter release at these receptor synapses[11]. In the locust the extracellular inhibition seems to be responsible for the narrowed receptive fields, implying higher acuity, which the lamina cells show over the receptors (ref. 13 and my unpublished work), also observed in the fly and dragonfly[18,19]. It would be an attractive scheme for use in complex systems of lateral interaction with many cell types, requiring as it does only a regular arrangement of neurones bisected by an extracellular barrier, to develop its specificity. The lack of anatomical connections and the disguised inverted sign of the inhibition in the soma (Fig. 2*b*) make it difficult to recognise, although it is of course well known at the axon cap of the Mauthner cell[20,21].

This work was begun at the Experimental Psychology Laboratory, University of Sussex. I thank Dr W. R. A. Muntz for facilities, and the SRC and Canadian NRC for support.

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Received February 24; accepted April, 17 1975.

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Influenza virus as a teratogen in rhesus monkeys

INFLUENZA virus has been suspected but never proven to be a cause of malformation of the central nervous system in man[1-4]. One major limiting factor in the evaluation of the teratogenic potential of this and other viral agents has been the absence of a primate model. So far the only studies on the teratogenic effects of this virus have been in the chick embryo and rodents, animals phylogenically distant from *Homo sapiens*[5-7]. We now report a primate model, the rhesus monkey, showing the teratogenic effect of influenza virus.

We inoculated the virus directly into the foetus, rather than into the mother, to ensure maximum opportunity for the virus to infect the foetus and cause damage[8]. Fourteen pregnant rhesus monkeys (*Macaca mulatta*), serologically negative for influenza A virus antibody, were subjected to laparotomy at 105–111 d gestation, this age having been suggested by the results of a pilot study. At this time influenza A virus was inoculated into the brain of the foetus by passing the inoculating needle through the intact uterine wall and the foetal anterior fontanelle. The inoculum was 0.25 ml (except for one animal, originally from the pilot study, which has received 0.5 ml) of an Aichi/2/68 WP strain of Hong Kong influenza A virus passaged fifteen times in egg allantoic sac and three times in rhesus kidney tissue culture. This virus titred $10^{6.7}$ egg infective dose 50% (egg ID_{50}). A further eleven pregnant monkeys without influenza A antibodies were inoculated similarly at 105–108 d gestation with control material: nine received 0.25 ml of the same virus pretreated with specific guinea pig antiserum against K/68AS/494 type strain in influenza A virus (neutralised virus), and two received 0.25 ml of control uninfected allantoic fluid.

Two foetuses inoculated with virus were delivered by caesarean section, respectively, 3 and 11 d after inoculation, and killed immediately. Virus was recovered from the brain, lung, liver, throat and amniotic fluid of both animals, showing that influenza virus replicated in rhesus monkey foetal tissues in our experimental conditions. Having established that infection occurred in the foetuses, we investigated its possible teratogenic effect.

The other 23 foetuses were allowed to reach term (160±5 d). Seven foetuses were delivered spontaneously (four infected and three controls): three of these were stillborn (two infected and one control). The other sixteen foetuses were delivered by caesarean section and all were liveborn (eight infected and eight controls). These animals were killed and examined: fourteen at 1 d (seven infected and seven controls) and one control at 3 d, whereas eight (five infected and three controls) were allowed to live 1 month to determine if any malformation would become more severe with time (this did not seem to be the case).

The virus was isolated in primary rhesus monkey kidney tissue cultures. Haemagglutination–inhibition antibody (HI) titres were determined by standard methods[9]. No virus was isolated from throat swabs taken at delivery from the mothers of either infected or control monkeys, suggesting the absence of persistent maternal infection. In six mothers whose foetuses had been inoculated *in utero* with virus, however, an antibody titre developed and persisted for the period of observation—9–15 months (serum was not available from the other six mothers)—indicating maternal infection. Virus was not recovered from the lung, thymus, spleen, kidney, liver, amniotic fluid or placenta of any of the foetuses. An attempt to isolate virus from the brain of three and the cerebrospinal fluid of eight infected foetuses was also negative. HI antibody titres against influenza A virus were, however, found in the serum of ten of the virus-inoculated foetuses (Table 1). We interpret this as evidence of active antibody production by the foetus, although passive transfer of antibody from the mother across the placenta cannot be excluded. Mothers and foetuses in the control group whose serum was available for testing, with one exception, were negative for influenza A antibody. The exception was an animal which had received incompletely neutralised virus (Table 1).

At necropsy the only macroscopically recognisable malformation was hydrocephalus, present in six of the 12 virus-inoculated animals (Table 1). It was manifest primarily as a

Table 1 Frequency of hydrocephalus and HI titres in rhesus monkey foetuses inoculated with influenza virus and in controls

Animal groups	No. hydrocephalic / No. observed	HI titre serum*
Infected group: 12 animals		
Virus inoculated	6/12	1:8–1:512
Control group: 11 animals		
Neutralised virus	0/8	<1:4
Partly neutralised virus	1/1	1:8
Allantoic fluid	0/2	—

*Serum was not available from two infected animals, one animal in the neutralised virus control group, and the two animals in the allantoic fluid control group.

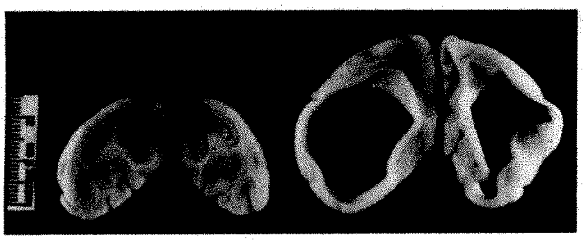

Fig. 1 Corresponding coronal sections of brains of 1-month-old rhesus monkeys after *in utero* intracerebral inoculation of influenza A virus at 107–108 d gestation. Brain on left, inoculated with neutralised virus, appears normal; brain on right, inoculated with virus alone, shows severe hydrocephalus bilaterally.

gross dilatation of the lateral ventricles that was bilateral in all but one case (Fig. 1). The dilatation was consistently most pronounced in the posterior horns of the lateral ventricles, and in one case was limited to a single posterior horn. Hydrocephalus did not occur in the two control animals inoculated with allantoic fluid. Of the nine controls inoculated with neutralised virus, one had hydrocephalus similar to that in the experimental group—unilateral, focal and moderate. This was the only animal inoculated with neutralised virus which developed an antibody titre (Table 1) and its mother had an antibody titre at delivery. This indicates incomplete neutralisation which is well known with influenza virus[10]. We believe that the hydrocephalus in this foetus was a sequela of infection with the viable virus in the partially neutralised inoculum.

Severe stenosis or total occlusion of the aqueduct of Sylvius, located between the third and fourth ventricles of the brain, has been reported to be the mechanism of neonatal hydrocephalus induced in the suckling hamster by myxovirus[7]. For this reason we examined the ventricular system of the brain in great detail in five of the six hydrocephalic animals in the infected group. Four had obstructive lesions in either the fourth or third ventricles, or the foramina of Monro, or in combinations. Obstruction of the aqueduct of Sylvius was not observed. On the basis of preliminary histological studies, the obstructive lesions could be classified as sequelae of a destructive ependymitis and/or choroiditis. The precise role of the obstructive lesions in the pathogenesis of the hydrocephalus, however, awaits further investigation.

Thus influenza virus can induce congenital hydrocephalus in the foetus of the rhesus monkey. There may be a similar teratological effect in the human in view of the close phylogenic relationship to subhuman primates. The demonstration that influenza virus can cross the human placenta and infect the foetus emphasises the need for caution in the use of live vaccines in the pregnant human female[11].

We thank Blanche Curfman, Renee Traub, and Robert Brown for technical help.

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Received March 10; accepted April 15, 1975.

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Non-encephalitogenic synthetic analogues of the determinant for allergic encephalomyelitis in guinea pigs

EXPERIMENTAL allergic encephalomyelitis (EAE) is an autoimmune demyelinating disease induced by sensitisation to whole central nervous system (CNS) tissue homogenates, to myelin basic protein (BP) or to peptide regions derived from BP[1–4]. One such region, peptide E, was isolated from the pepsin digest of the BP and induces EAE in guinea pigs[3,5]. The shortest segment of peptide E which induces EAE, H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-OH (peptide S3), has been synthesised by the Merrifield procedure[6]. Studies with analogous synthetic peptides with single amino acid residue substitutions have shown that the linearly spaced array of tryptophan, glutamine and lysine is needed to induce EAE[6]. We have now determined the minimum snbstitution in the amino acid sequence of peptide S3 necessary to destroy encephalitogenicity and to preserve the sequence for delayed hypersensitivity (DTH), also reported for peptide S3[7]. The sequence requirement for induction of EAE is not restricted to tryptophan, glutamine and lysine, and certain analogous peptides retain the ability to induce and elicit DTH unaccompanied by signs of EAE.

All peptides were synthesised from Boc–glycine–resin ester containing 0.25–0.50 μmol amino acid per g resin (Schwarz/Mann) using an adaptation of the Merrifield solid phase method[8]. Details of the synthesis of peptide S3 and its analogues, including methods of coupling, cleavage and purification have been described before[9]. High voltage electrophoresis or paper chromatography of each purified peptide showed a single spot with ninhydrin stain. Amino acid analyses of samples hydrolysed at 110 °C for 24 h with 6N HCl gave values close to whole integers of amino acids expected for each particular sequence.

All EAE assays were carried out in Rockefeller–Hartley guinea pigs (500–600 g). Each peptide was tested in doses of 33.3–1,000 μg, as was the encephalitogenic BP from bovine myelin. Each antigen was administered in a single injection of 0.1 ml, made up in saline emulsified with an equal volume of Freund's complete adjuvant (FCA) (Difco). Animals that developed severe clinical signs of EAE were killed immediately and all others were killed after 30 d. Brain and spinal cord tissues were examined histologically for lesions characteristic of EAE[2].

The DTH assay was performed on day 11 after sensitisation. The skin test antigen was administered intracutaneously in 0.1 ml of sterile saline as described previously[7], and the injection site was inspected after 1, 3, 4 and 24 h. The cross sectional diameter of the erythematous area was recorded after 24 h.

The amino acid sequences of the peptides used are shown in Table 1 and the EAE and DTH assays are summarised in Table 2. The presence of lesions in animals receiving 33.3 μg and 100 μg corresponded with the clinical manifestation of the disease.

The DTH skin response to 0.10 μmol of each peptide antigen ranged between 9×10 and 12×13 mm. Positive skin tests were confirmed histologically. Thin sections of the injected site together with adjacent areas showed extensive plasma cell infiltrates indistinguishable from the delayed skin response to BP. Neither the delayed skin response nor the size of the erythematous area differed significantly when guinea pigs were sensitised and skin tested with the BP or any antigen listed in Table 1. Ovalbumin and bovine serum albumin, used for control skin test antigens, gave negative results.

Our results confirm that phenylalanine or valine substituted

Table 1 Amino acid sequence of synthetic peptides

Laboratory code no.		1 2 3 4 5 6 7 8 9 10	No. of substituted residues
1	S3 (peptide E)	H-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Gly-OH	None
2	S31	H-Phe-Ser-Phe-Gly-Ala-Glu-Gly-Gln-Lys-Gly-OH	3
3	S37	H-Phe-Ser-Val-Gly-Ala-Glu-Gly-Gln-Lys-Gly-OH	3
4	S38	H-Gly-Gly-Trp-Gly-Ala-Glu-Gly-Gln-Lys-Gly-OH	1, 3
5	S35	H-Ala-Ser-Trp-Gly-Gly-Gly-Gly-Gln-Lys-Gly-OH	1, 5, 6
6	S23	H-Phe-Ser-Trp-Gly-Ser-Leu-Pro-Gln-Lys-Gly-OH	5, 6, 7
7	S25	H-Phe-Ser-Tyr-Gly-Ala-Glu-Gly-Gly-Lys-Gly-OH	3

The amino acid sequence of synthetic peptides used in the study are shown. From 150–225 mg of each peptide antigen were prepared. The recovery ranged from 40–60% of theoretical yield.

for tryptophan destroys encephalitogenicity[6], and have shown that tyrosine substituted for tryptophan renders the peptide EAE-inactive. This underlines the importance of the tryptophyl residue in preserving the encephalitogenic function of the peptide. But this role of tryptophan can be emphasised only in relationship to other amino acids of the sequence of peptide S3. The presence of tryptophan in its required position in the sequence, accompanied by glycine-glycine substituted for phenylalanine-serine (peptide S38) or glycine-glycine substituted for alanine-glutamic acid (peptide S35) did not retain encephalitogenicity. Peptide S23 contained tryptophan, glutamine, and lysine at their native positions, but other substitutions destroyed its encephalitogenicity.

It is interesting that tyrosine is an essential component of a structurally similar determinant, H-Thr-Thr-His-Tyr-Gly-Ser-Leu-Pro-Gln-Lys-OH, (residues 65–74 of the BP molecule[10]) which induces EAE in rabbits[4]. Tyrosine is at a position identical to that of tryptophan, separated by four residues from the C-terminal sequence of glutamine-lysine. The nature of these four residues is important because peptide S23, with serine-leucine-proline substitutions at positions five, six and seven and intact N- and C-terminal sequences, did not retain the activity of peptide S3. Thus each component amino acid has a role in the activity of peptide S3. The requirement for tryptophan in the encephalitogenic sequence cannot be attributed to the aromatic nature of its side chain, since phenylalanine or tyrosine substitutions, as in peptides S31 and S25 respectively, did not fulfil the requirement for encephalitogenicity. Thus the determinant has a unique chemical structure, and any amino acid substitution alters its biological function.

Although amino acid substitutions destroyed the encephalitogenicity of the determinant, the DTH response was not abolished. The peptides retained the capacity to induce and elicit DTH responses in guinea pigs sensitised with encephalitogenic or non-encephalitogenic antigens. The preparation of non-encephalitogenic analogues of the EAE-inducing determinant bearing structural specificity recognised by animals sensitised with BP or peptide S3 provides antigens for the study of prevention, suppression and treatment of EAE.

Table 2 Biological properties of synthetic analogues of the determinant for allergic encephalomyelitis in guinea pigs

Sensitising antigen dose	33.3	100 (μg in FCA)	333	1,000	DTH
S3 (peptide E)	10/13	5/5	2/8	3/8	+
S31	0/8	0/16	0/4	0/4	+
S37	0/5	—	0/5	0/5	+
S38	0/5	0/5	0/10	0/10	+
S35	0/4	—	0/4	0/4	+
S23	0/5	—	0/5	0/5	+
S25	0/5	0/4	0/5	0/5	+
Bovine basic protein	15/18	16/21	4/4	4/4	+

The sensitising antigens were injected in 0.1 ml FCA emulsion at the above concentrations in the hind foot pad. Sensitised animals were skin-tested for delayed type hypersensitivity (DTH) at day 11 after sensitisation. The lowest dose of peptide S3 and bovine basic protein was 25 μg rather than 33.3 μg.

The work was supported by grants from the Continental Corporation Foundation, the Clark Foundation and the National Multiple Sclerosis Society.

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Inhibition of leukocyte locomotion and chemotaxis by lipid-specific bacterial toxins

SINCE a number of cytolytic bacterial protein toxins have specific actions on individual membrane lipids, these toxins, used at sub-toxic doses, should be useful for exploring the role of lipids in membrane-activated cell functions. I have suggested[1-3] that leukocyte chemotactic factors initiate a locomotor response by virtue of their ability to penetrate the hydrophobic interior of the lipid bilayer of the cell membrane, and thus possibly to bring about changes in the intracytoplasmic concentration of divalent cations and activation of contractile systems in the cytoplasm. If this is so, it may be possible to modify locomotor and chemotactic responses in leukocytes using lipid-specific toxins. The oxygen-labile lysins such as streptolysin 0 or *Clostridium perfringens* θ toxin show a specific affinity for cell membrane cholesterol. Bacterial phospholipases C attack the polar heads of a number of membrane phospholipids[4]. Among those phospholipases, sphingomyelinase C, the β toxin of *Staphylococcus aureus*, shows a high substrate specificity for sphingomyelin[5]. Here I show that the locomotor responses of leukocytes to chemoattractants are modified by these toxins and that neutrophils show a different pattern of response from monocytes. The locomotor response of human blood monocytes is inhibited by *Cl. perfringens* phospholipase C (PLC) and by sphingomyelinase C but not by *Cl. perfringens* θ toxin. In contrast, the response of human blood neutrophils is inhibited by θ toxin but not by phospholipases C.

Human blood neutrophils and monocytes were obtained from normal venous blood and separated from one another by centrifugation on Ficoll–triosil after preliminary sedimentation of the red cells with dextran. Leukocyte locomotion and chemotaxis were measured by the micropore filter method as described previously[6] by determining the distance migrated by the leading front of cells[7]. The distinction between oriented and non-oriented migration was made by varying the concentrations of chemotactic factor above and below the filter and calculating the deviation from expected random migration as described by Zigmond and Hirsch[7]. The toxins were pure[8] and not contaminated with one another; an important point since commercial samples of PLC which have been reported to influence leukocyte function, may contain θ toxin[9], and commercial PLC had θ-like effects in the experiments reported here. Sphingomyelinase C was prepared from *Staphylococcus aureus*[10] and contained a trace of nuclease which does not affect chemotaxis (P. C. W., unpublished). The viability of leukocytes after toxin treatment was checked by direct observation of morphological changes and by Trypan blue exclusion. No cytotoxic effects were seen at doses of PLC below 400 mU ml^{-1}, of θ toxin below 1 haemolytic unit (HU) ml^{-1} or of sphingomyelinase C at any dose tested ($<10^4$ HU ml^{-1}).

The toxins had little effect on unstimulated random leukocyte locomotion but they inhibited the ability of leukocytes to respond to the presence of a chemotactic factor by increasing the speed of their locomotion. This inhibition was seen not only when cells were migrating in a chemotactic gradient but also when the concentration of the factor was uniform throughout the system, which suggests that the toxins act on the mechanism of locomotion itself rather than on the mechanism by which a cell becomes orientated in a gradient. The effect of PLC and of θ toxin on the migration of human neutrophils towards casein at varying doses is shown in Fig. 1*a*. θ Toxin inhibited the migration of neutrophils towards casein and other chemotactic factors such as endotoxin-activated serum and alkali-de-natured human serum albumin (HSA). In contrast, PLC had no inhibitory effect on neutrophil locomotion (Fig. 1*a*). Addition of free cholesterol to the medium prevented the inhibitory action of θ toxin on neutrophil locomotion (Table 1).

The effect of the same toxins on migration of human blood monocytes is shown in Fig. 1*b*. PLC inhibited the migration of monocytes towards casein but θ toxin at sub-toxic doses had no effect. These results were thus the opposite of those obtained with neutrophils. PLC slightly inhibited the migration of unstimulated monocytes but inhibited the migration of chemically stimulated cells more strongly. The effects of sphingomyelinase C resembled those of PLC (Fig. 1*c*). It inhibited the response of monocytes to casein or to alkali-denatured HSA at doses of enzyme down to 1 HU ml^{-1} but did not inhibit monocyte migration in the absence of a chemotactic factor. Sphingomyelinase C was without effect on neutrophil locomotion.

The fact that lipid-specific bacterial toxins inhibit the chemically directed locomotion of leukocytes supports the hypothesis that interactions of chemotactic factors with membrane lipids are of importance in initiating this locomotion. The differences in responsiveness between neutrophils and monocytes raise some interesting questions. θ toxin, like streptolysin O[11], another cholesterol binding toxin, inhibits the response of neutrophils but not monocytes to chemotactic factors, which suggests that cell membrane cholesterol may function in the

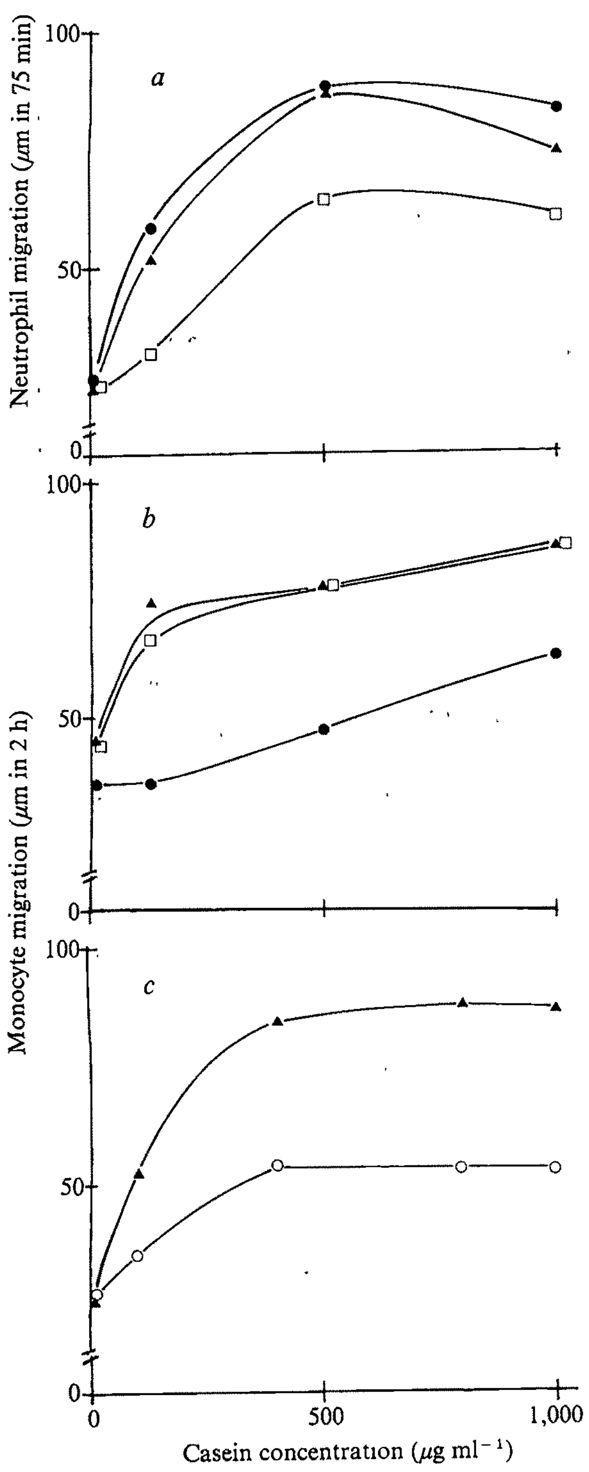

Fig. 1 *a*, Dose–response curves of human neutrophils to casein in the presence of *Cl. perfringens* PLC (200 mU ml^{-1}) (●), or θ toxin (0.3 HU ml^{-1}) (□), or in the absence of added toxin (▲). PLC activity was determined by Dr R. Mollby as described previously[8]. θ Toxin was reduced with 2-mercaptoethanol and assayed by haemolytic titration against 1% sheep cells. Toxins were added to the chemotactic chambers at equal concentrations on both sides of the filter immediately before incubation. In other experiments leukocytes were reincubated with toxin then washed. Inhibition similar to that shown in the figure was observed. *b*, Dose–response curves of human monocytes to casein in the presence of PLC (200 mU ml^{-1}). (●), θ toxin (0.3 HU ml^{-1}). (□), or untreated (▲). Conditions of assay as in *a*. Monocytes preincubated with PLC then washed free of toxin also showed inhibition of locomotion to casein. *c*, Dose–response curves of human monocytes in the presence (○), and absence (▲) of sphingomyelinase C (400 HU ml^{-1}). Conditions of assay as in *a*. The toxin was assayed by hot–cold haemolysis[15] by Dr Christine McCartney. Fig. 1*a–c* shows leukocyte migration in a positive gradient, that is, from zero concentration of casein above the filter to the stated concentration below it.

Table 1 Effect of cholesterol on the inhibition of human blood neutrophil migration by θ toxin

	Neutrophil migration (μm in 75 min) No casein	Casein 800 μg ml⁻¹ below filter
Untreated neutrophils	25	99
Neutrophils treated with θ toxin 0.3 HU ml^{-1}	24	52
Neutrophils treated with θ toxin 0.3 HU ml^{-1} and cholesterol 10^{-4} M		95

interaction of such factors with neutrophils but less so with monocytes. The inhibition of monocyte migration obtained with sphingomyelinase C suggests that in the monocyte the interaction involves sphingomyelin. It is probably relevant that, from published data for human[12] and other[13] cells, the molar ratios of cholesterol to sphingomyelin and of cholesterol to phosphatidylcholine are about twice as high in neutrophil membranes as in those of monocytes or macrophages, but it will not be possible to be more precise about these interactions until more is known about the lipid composition of human leukocyte plasma membranes and how these lipids interact with proteins. There may be species differences too, since the inhibitory effect of streptolysin 0 on neutrophil chemotaxis varies from species to species[14]. The actions of membrane-active bacterial toxins of good purity on other leukocyte functions require further study. These proteins should prove useful not only as probes of the role of individual lipids in membrane function; their effects are also pertinent to the infective process in bacterial disease.

I thank Drs Christine McCartney, J. P. Arbuthnott, C. Smyth and R. Möllby for gifts of toxins and for advice, and the MRC for financial assistance.

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Received March 21; accepted April 16, 1975.

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Suppression of tumour growth in mice by a drug–antibody conjugate using a novel approach to linkage

The possibility of using cytotoxic drugs linked to tumour-specific antibodies as a form of cancer chemotherapy has been revived recently. In theory the antibodies would selectively transport the drugs to the target tumour site. The demonstration by Ghose *et al.*[1,2] and also by Flechner[3] that chlorambucil and antibody combined produce an augmented antitumour effect can, however, be explained by an additive or synergistic effect of drugs and antibody uncombined, since there is a high probability of *in vivo* dissociation[4–6]. Although this synergistic drug–antibody effect has been studied in detail[7–9], the original aim of preparing a stable drug–antibody conjugate has also been pursued and evidence of *in vivo* tumour suppression has been obtained with covalent conjugates of rabbit antitumour immunoglobulin (Ig) and alkylating agents[10,11]. In these studies, direct conjugation of the drugs with Ig was carried out using a water-soluble carbodiimide. Successful tumour suppression was, however, dependent on using large amounts of linked material since severe limitations are imposed on the degree of drug substitution by the physicochemical changes induced in the immunoglobulin by linkage. These changes are reflected in loss of antibody activity and poor water solubility of the preparations thus limiting the clinical potential of complexes in this form. Here we describe a method of overcoming these problems. Instead of direct substitution of the drugs on the Ig, an intermediate carrier molecule is heavily substituted and the drug carrier then linked to Ig. By minimising interference with the chemical structure of Ig in this linkage step, a conjugate is produced with both a high concentration of drug and little loss of antibody activity. This conjugate has proved effective in suppressing tumour growth in mice.

It was planned to attach drug to carrier by means of a carbodiimide induced carboxyamide bond and therefore suitable compounds were sought containing free carboxyl or free amino groups. Since many polycations are toxic[12], a polyanion —polyglutamic acid (PGA)—was chosen as the carrier and *p*-phenylenediamine mustard (PDM), with its free amino group as the drug. The PGA was poly-L-α-glutamic acid, molecular weight, 35,000 (Miles Seravac) and the PDM, N,N-*bis*-(2-chloroethyl)-*p*-phenylenediamine, was supplied by the Chemical Defence Establishment, Porton. The drug–carrier complex PDM–PGA was prepared by reacting 250 mg of PGA in aqueous solution at 10 mg ml^{-1} with 1 g EDC (1-ethyl-3(3-dimethylaminopropyl)carbodiimide) and 14 ml of PDM (10 mg ml^{-1}) at room temperature. The PDM–PGA was isolated by precipitation and washing at *p*H 4.0 in the cold, and finally redissolved by addition of 1 N NaOH. Evidence of a covalent conjugate came from a shift in the ultraviolet absorption spectrum of PDM after linkage from $\lambda\text{max}_{258\,\text{nm}}$ to $\lambda\text{max}_{277\,\text{nm}}$ and from various tests showing that the PDM and PGA behaved as one entity in conditions of dialysis, gel filtration and ion-exchange chromatography. Determination of alkylating activity of the PDM–PGA was by a modification of the Epstein[13] test in which the reaction temperature was reduced to 80 °C. From this test and results of ultraviolet spectroscopy the substitution ratio of mol PDM per mol PGA was calculated to be 45:1, representing a substitution of approximately 16% of the available carboxyl groups of PGA.

PDM–PGA was coupled to Ig from a rabbit antiserum against mouse lymphoma cells (EL4) made specific by repeated absorption with normal mouse spleen cells[14]. Linkage was effected by a carbodiimide reaction between free carboxyl groups on the drug carrier and amino groups on the Ig using the following ‘concentration-dilution’ technique. PDM–PGA was concentrated to 200 mg ml^{-1} by membrane ultrafiltration and EDC added to a final concentration of 40 mg ml^{-1}. After 2 min at room temperature this mixture was diluted 40-fold with phosphate-buffered saline (*p*H 7.2) and added to an equal volume of Ig at 5 mg ml^{-1}. Excess EDC was quenched by addtion of sodium acetate to a final concentration of 0.15 M. The mixture was dialysed against cold saline to remove unwanted low molecular weight components and finally reconcentrated by membrane ultrafiltration. Analysis of the complex gave values for PDM–PGA–Ig of 2:8:10 mg ml^{-1} respectively. A variety of physicochemical tests were carried out to show that coupling had taken place. These include ethanol precipitability, ion-exchange chromatography and immunoelectrophoresis. Antibody activity of the complex was determined by a ^{51}Cr-release microcytotoxicity assay[14]. EL4

Table 1 Survival of C57BL/6 mice bearing EL4 lymphoma

Daily treatment (intraperitoneal) (days 1–4)	Median survival time* (d)	Mice alive free of tumour at day 60
0.4 ml saline	13	0/5
0.4 ml Ig (4 mg)	19	0/5
0.4 ml PDM–PGA (0.75 mg PDM)	25	1/5
0.4 ml Ig (4 mg)+0.4 ml PDM–PGA (0.75 mg PDM)	38	2/5
0.4 ml PDM–PGA–Ig (0.75 mg PDM, 4 mg Ig)	>100	5/5

*Median survival time as defined in ref. 16 is the time to which 50% of the animals in the group survive.

cells were used as targets and were incubated for 3 h with guinea pig serum as complement source. C57BL/6 lymph node cells (strain of origin of tumour) were used as a control for tumour specificity. From such tests it was shown that 66% of the original anti-EL4 activity was retained in the complex and there was no loss of specificity.

To show the effectiveness of the complex as an antitumour agent *in vivo*, several groups of five C57BL/6 mice were inoculated with 5×10^4 live EL4 cells intraperitoneally. This is approximately 10,000 times the LD_{50} challenge dose. After 24 h the mice received the first of four daily injections of the complex or control preparations. Table 1 shows the results of such an experiment. A small increase in survival time was obtained with the Ig alone and with the PDM–PGA alone. When PDM–PGA and Ig were given together, uncombined, the mice survived nearly three times as long as the mice treated with saline—a drug–antibody effect. The greatest increase in survival was obtained, however, when the complex was given, no mice having died by day 60.

PDM has also been conjugated to glutamyl-containing synthetic polypeptides by carbodiimides in a study of the use of macromolecules as carriers for cytotoxic groups[15]. In those experiments although the toxicity of the PDM was markedly reduced on conjugation, little improvement in therapeutic efficiency was obtained. No attempt was made to direct the drug to the tumour target by linking those preparations to tumour-specific antibody. In our study, PDM conjugated to PGA, produces a material with a similarly reduced toxicity. (LD_{50} of ~200 compared with LD_{50} of 5 for free PDM.) This material on linkage to Ig containing tumour-specific antibody produced a complex with powerful antitumour properties. The greater effectiveness of the complex compared with the PDM–PGA plus Ig unlinked, provides evidence that the complex is stable and suggests that a homing mechanism and not a drug–antibody effect is operating.

How such a complex exerts its cytotoxic action is not yet known. It may be sufficient to alkylate cell surface components for cell death to occur. Alternatively, enzymes associated with the cell membrane could release the drug from the conjugate and allow entry of free drug into the cell. Another possibility is that the entire conjugate is pinocytosed to exert its action within the cell.

The concept of linking cytotoxic drugs to antibody through an inert intermediate carrier offers a wide scope for improved cancer chemotherapy in the future, with the possibility of using a variety of drugs, different carriers and antibody preparations of greater purity.

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Received February 20; accepted April 28, 1975.

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Inhibition of murine plasmacytoma TEPC 15 by its specific antigen

In favourable conditions hosts can produce antibodies to components of their tumours. An intriguing example is the ability of BALB/c mice immunised with syngeneic myeloma proteins MOPC 315 or MOPC 460 (ref. 1) to make antibodies to the idiotypic determinants of these proteins. The sensitised mice reject tumour-producing MOPC 315 or MOPC 460 cells; this may result from reaction of anti-idiotypic antibodies with myeloma proteins on myeloma cell surfaces. On the assumption that any substance able to bind to a cell membrane surface component could modify the rate of cell division, we hoped to inhibit myeloma growth by injecting specific antigen into the hosts. This plan was based on the observation that specific antigen binds to the cell surface of antibody-secreting myeloma cells[2]. We describe here the inhibition of the growth of plasmacytoma TEPC 15 by the specific pneumococcus C polysaccharide (PCP) antigen.

Murine plasmacytomas are transplantable in mice and secrete a specific clonal immunoglobulin. TEPC 15 excretes an IgA immunoglobulin which specifically binds PCP[3]. MOPC 315 excretes another IgA immunoglobulin which binds both dinitrophenyl (DNP) and trinitrophenyl groups[4]. Both tumours were adapted to grow as ascites cells after intraperitoneal injection; they also induced tumours in the peritoneum. Both tumours have membrane-bound immunoglobulin with the same respective antigen specificities.

PCP after purification[5,6] contained phosphorylcholine and could thus be considered a specific antigen (and phosphorocholine the specific hapten) for TEPC 15. Conversely, we used DNP-bovine gamma globulin (BGG-DNP, 38 residues per BGG molecule) as the specific antigen (and 2,4-dinitrophenol as specific hapten) for MOPC 315. Ascites cells were obtained in 8–12-week-old SJBALB/c mice by one intraperitoneal injection of 5×10^4 cells for TEPC 15 and 3×10^4 cells for MOPC 315. These tumour cells were obtained in ascitic fluid in densities of $2\text{–}20\times10^6$ ml^{-1}, with a viability of 90–98% as tested by trypan blue exclusion. Fewer cells of MOPC 315 than TEPC 15 were needed to induce ascites and tumours, and the former grew much faster than the latter. In most cases we injected cells and

Table 1 TEPC ascites in mice treated twice a week

Injection	Treatment	Mice with tumours 4th week	6th week
5×10^4 cells	Saline	20/25	22/25
	10 μg PCP	7/20	15/25
	10 μg Dextran	9/15	12/15
	10 μg BGG-DNP	9/10	10/10

Mice were injected intraperitoneally with 5×10^4 cells in 0.2 ml and received either saline for controls or antigen treatment in 0.2 ml by the same route. Then twice a week they again received intraperitoneally, the same treatment without cells. The mice were killed and autopsied on week 4 for one experiment, and on week 6 for the other. Mice without tumours or ascites are recorded as 0; mice with either tumour or ascites are recorded as 1.

antigen or hapten intraperitoneally on day 1. Subsequently, we injected the antigen or the hapten twice a week. Mice were killed for necropsy at the times shown in the legend to Table 1.

Table 1 shows that, whereas 20 out of 25 control mice had ascites and tumours by week 4 and 22 of them had lesions by week 6, only 7 out of 20 receiving 10 μg of PCP twice a week developed lesions. Results not shown here indicate that this effect is dose responsive above the minimal concentration of 1 μg. This inhibition of tumour growth could be the result of adjuvant-like activity. Dextran which is also a polysaccharide, and known to be an adjuvant[7], was tested for anti-tumour activity. Table 1 shows that tumour development in mice treated with dextran was no different from that in controls.

BGG-DNP (the specific antigen for MOPC 315) had no effect on the growth of TEPC 15, but it did inhibit growth of MOPC 315 (Table 2). Although 11 out of 12 control mice had large tumours after 2 weeks, only eight out of 15 treated with BGG-DNP had lesions, and 14 out of 15 treated with PCP had lesions. Moreover, the lesions found in the animals given BGG-DNP were much smaller than the others. The situation was similar on week 4. Whereas specific antigens had a specific inhibitory effect, the related haptens had no effect although we used large doses: neither phosphorylcholine nor dinitrophenol slowed the growth of TEPC 15 or MOPC 315 (Table 3).

Table 2 MOPC 315 ascites in mice treated once a week

Injection	Treatment	Mice with tumours (size of lesions) 2nd week	Mice with tumours (size of lesions) 4th week
2×10^4 cells	Saline	11/12 (Large tumours)	12/12 (Large tumours)
	10 μg BGG-DNP	8/15 (Small tumours)	12/12 (6 Small tumours 6 Large tumours)
	10 μg PCP	14/15 (Large tumours)	15/15 (Large tumours)

As in Table 1, antigens were injected once a week. Large tumours had mean diameters >6mm. Small tumours had mean diameters <2mm.

Table 3 Comparative activities of specific haptens and antigens on ascites growth

Plasmacytomas	Treatment	Mice with tumours Week 2	Mice with tumours Week 4
TEPC 15 5×10^4 cells	Saline	5/10	9/10
TEPC 15 5×10^4 cells	2 μg Phosphorylchloline	4/5	5/5
TEPC 15 5×10^4 cells	10 μg PCP	1/10	5/10
MOPC 315 2×10^4 cells	Saline	11/12	11/12
MOPC 315 2×10^4 cells	10 μg DNP	11/11	9/9
MOPC 315 2×10^4 cells	10 μg BGG-DNP	6/14	9/13

As in Table 1, antigens were injected twice a week.

We do not know the mechanism of this inhibitory effect. The host immune system does not seem to be involved because at such high doses, pneumococcal polysaccharides are tolerogenic[8,9], even in a single dose[9]. Thus we favour a direct effect of the antigen on tumour cells at the membrane level. Because the IgA immunoglobulins secreted by both plasmacytomas are unable to bind complement, we assume that the inhibitory effect is not mediated by the complement. Concanavalin A has been shown to bind to the B lymphocyte cell membrane, resulting in capping and leading ultimately to blastogenesis and synthesis of IgM[11,12]. Antigens also induce capping and blastogenesis in immunogenic doses, whereas such phenomena are inhibited by tolerogenic doses[13]. Furthermore, low doses of mitogens can stimulate the membrane Na^+,K^+-stimulated ATPase[14,15] and 5′-nucleotidase activity, although the latter is inhibited by high doses[16]. Thus we suggest that the PCP antigen sticks to the tumour cell surface, leading to structural and enzymatic changes in the cell membrane which in turn inhibit cell division.

We thank Mrs Delessert for technical assistance. We thank Dr M. Potter for plasmacytoma tumours, Dr E. Gottschlisch for purified PCP and Dr J. J. Metzger for preparing BGG-DNP. We also thank them and Drs M. Kehoe and J. Sheppard for constructive suggestions. This work was supported in part by a contract from la Délégation Générale à la Recherche Scientifique and by a grant from NATO and by the Fondation pour la Recherche Médicale Française.

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Suppressor T cells in mice made unresponsive to skin allografts

WE have established[1] that long-lasting, strain-specific unresponsiveness to H-2 incompatible skin allografts can be induced in 30–50% of adult CBA mice by pretreatment with a single injection of a crude extract of liver from the graft donor strain, combined with a dose of *Bordetella pertussis* vaccine and a short course of antilymphocyte serum (ALS). Lymphoid cells removed from mice bearing healthy well-established allografts were reactive against graft donor strain antigens when tested in mixed lymphocyte culture (MLC), cell-mediated lympholysis tests (CML) and in graft *versus* host assays (GvH)[2]. Haemagglutinating and cytotoxic antibodies specific for the donor strain were invariably absent from the serum of unresponsive mice although small quantities of IgG1 antibodies have been detected by a more sensitive technique[3]. We have so far been unable to detect any strain-specific suppressive activity in sera using MLC, CML and GvH tests[2] or when passively transferring sera to skin-grafted mice.

In view of increasing evidence for the existence of suppressor T cells (reviewed in refs 4–6) we have investigated the possibility that they may be important in maintaining the unresponsiveness in our mice. The experiments reported here suggest that they are important in this respect, but it would be premature to infer that suppressor T cells provide the only mechanism for the maintenance of this unresponsiveness.

Spleens were removed from unresponsive CBA mice bearing healthy A strain (= A/Jax) skin grafts 90–200 d after transplantation, and cell suspensions were made in phosphate-buffered Ringer solution with or without 5% foetal calf serum. Spleen cells were injected (half intravenously, half intraperi-

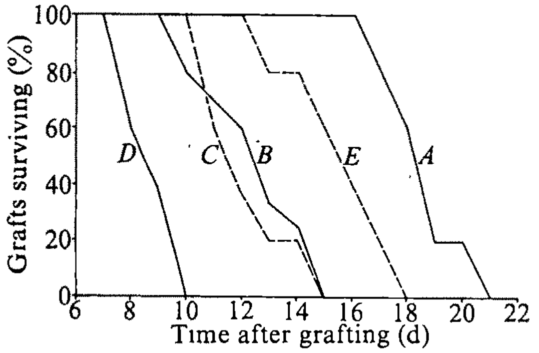


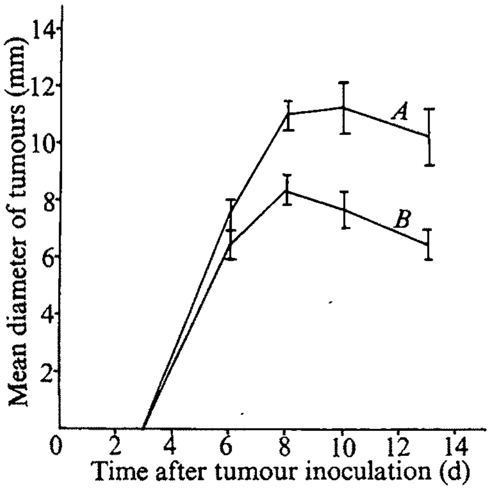
Fig. 1 Adoptive transfer of 2×10^8 unresponsive CBA spleen cells to normal syngeneic mice: effect on survival of A strain skin grafts. Recipients were also treated with serum from their spleen cell donors (see text). *A*, Unresponsive cells (five mice); *B*, no treatment (11 mice); *C*, normal cells (five mice); *D*, sensitised cells (ten mice); *E*, unresponsive cells (separate experiment) without serum (five mice).

toneally) into normal or ALS-treated syngeneic recipients which were challenged with A strain skin grafts or injected subcutaneously with A strain sarcoma 1 tumour cells.

Spleen cells (2×10^8) from unresponsive mice were injected into normal CBA males 3 d before grafting with A strain skin. Control mice received skin grafts only or were injected with 2×10^8 spleen cells from normal mice; another group received cells from sensitised mice that had recently rejected A strain skin grafts. In this experiment the mice were treated, in addition, with serum from their spleen cell donors, and were given 0.5 ml intraperitoneally on the day of grafting and 0.2 ml on the following day. Figure 1 shows that the recipients of 'unresponsive' cells (group *A*) retained their grafts significantly longer than controls (groups *B* and *C*). Recipients of sensitised cells (group *D*) exhibited accelerated rejection. In a separate experiment the adoptive transfer of 2×10^8 spleen cells from unresponsive mice, without additional serum, also prolonged graft survival, though less markedly (group *E*). We subsequently observed that the passive transfer of as much as 1.5 ml of serum from unresponsive mice, without cells, did not affect the survival of A strain skin grafts in the slightest.

CBA female mice which had been injected with unresponsive spleen cells showed impaired ability to reject A strain sarcoma 1 tumours (Fig. 2). Viable spleen cells (1.4×10^8) from unresponsive or normal mice were injected into CBA females, and on the same day, 10^6 sarcoma 1 cells were inoculated subcutaneously into the flank. Figure 2 shows that tumour rejection was significantly suppressed in the recipients of unresponsive cells (group A) compared with controls (group *B*). Tumours in both groups were eventually rejected but after day 13 ulceration of the tumours prevented precise measurements.

Fig. 2 Adoptive transfer of 1.4×10^8 unresponsive CBA spleen cells to normal syngeneic mice: effect on growth of A strain sarcoma 1. Tumour growth in mice injected with (*A*) unresponsive cells (*B*) normal cells (five mice per group, mean diameters of tumours are plotted ± s.e.).



To determine whether T cells were required for the successful transfer of unresponsiveness, spleen cells from unresponsive mice were treated with anti-θ antibodies and complement *in vitro* before transfer. One hundred million viable cells or the same dose reduced to 55×10^6 viable cells by treatment with anti-θ and complement, were injected into CBA male mice which had been grafted with A strain skin 10 d before and which had received a short postoperative course of ALS. Figure 3 shows that the survival of skin grafts was considerably improved by injecting untreated unresponsive cells (groups *A* and *E*) or those treated with complement only (group *B*), whereas pretreatment with anti-θ and complement (group *C*) abolished this effect and even curtailed graft survival compared with the ALS controls (group *E*). Normal spleen cells too (group *D*) hastened rejection.

These experiments and others[7] suggest that reactivity in mice retaining skin allografts for long periods in this model is under active restraint. Our data provide no direct evidence for the specificity of transferred unresponsiveness, although unresponsiveness in the spleen cell donors was exquisitely strain specific (L.B. and P.J.K., unpublished). That treatment with anti-θ antibodies and complement abolished suppressor activity from unresponsive spleen cells clearly indicates a role for T cells but it does not distinguish between a direct immunosuppressive effect of T cells and their possible cooperation with B cells (either in the transferred donor population or in the host) in the production of blocking antibodies. The detection of only small quantities of IgG1 antibodies in the serum of spleen cell donors[3] and the failure of serum to suppress either *in vitro* or *in vivo* reactivity[2], however, suggest that serum-blocking factors are unlikely to be involved. At present the production of specific suppressor substances directly from T cells[8,9] is a more plausible possibility. It is of interest that evidence has recently been presented that suppressor cells may be important in tolerance induced by injection of allogeneic cells into embryos[10] or neonates[11].

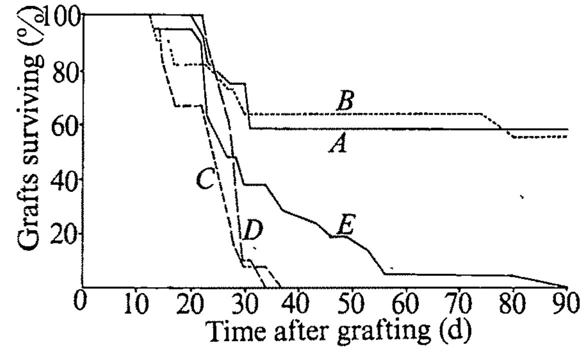


Fig. 3 Treatment of unresponsive CBA spleen cells with anti-θ and complement: effect on the adoptive transfer into ALS-treated secondary hosts of unresponsiveness to A strain skin grafts. ALS (0.5 ml) given on days 1, 3 and 6 after skin grafting, cell transfer on day 10 (10–12 mice per group). *A*, Untreated unresponsive cells; *B*, cells treated with complement only; *C*, cells treated with anti-θ and complement; *D*, cells from normal mice; *E*, ALS only.

Confirmation of the data reported here has been found in other transfer experiments using sublethally irradiated as well as ALS-treated secondary recipients (ref. 7 and L.B. and P.J.K., unpublished). We have, however, found considerable variability in the level of unresponsiveness transferred, but purely technical reasons may be responsible.

An anomaly that emerges from our investigation is that spleen cells from unresponsive mice were reactive against graft antigens in MLC, CML and GvH, but suppressed reactivity against skin grafts when transferred to syngeneic recipients. It is conceivable that the dose of antigen and its presentation may determine whether reactivity or suppression predominates and that the great excess of antigen present in the MLC, CML and GvH assays may circumvent or overwhelm the suppressor mechanism.

We thank Mr R. Butler, Mrs A. V. Carlson and Miss J. Parmar for assistance and the MRC for financial support.

Note added in proof: We now have evidence that purified T cell suspensions can transfer unresponsiveness to ALS-treated recipients. In another experimental model, Diener and his colleagues[12] have demonstrated the presence of suppressor cells in the lymph nodes of mice unresponsive to heart allografts.

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Received February 14; accepted April 22, 1975.

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Functional fractionation of human cytotoxic cells using differences in their cation requirements

Demonstration of anti-tumour immunity in man is often impaired by the variable level of cytotoxicity of normal control cell populations. Using physical fractionation techniques, attempts have been made to remove selectively the cells which are responsible for this 'variable background' effect[1,2], and to improve characterisation of cytotoxic cell populations. We report here that several types of human cell-mediated cytolysis have different extracellular cation requirements either for Ca^{2+}, for Mg^{2+}, for both, or for neither. These findings suggest the existence of differences in the mechanism(s) of lysis, and lead us to propose an alternative, functional approach to the fractionation of cytotoxic cells.

Four different systems were investigated with human peripheral blood cells as effector cells: anti-allogeneically sensitised cells (thymus-derived, T, cytotoxic cells[3]) against lymphocytes transformed with phytohaemagglutinin (PHA blasts); normal cells against either antibody-coated erythrocytes or antibody-coated tumour cells (non-T cytotoxic cells in both instances[3,4]); uncoated tumour cells.

Cytolysis of PHA blasts by *in vitro* sensitised anti-allogeneic human cells required Ca^{2+} (Fig. 1*c*). This Ca^{2+} dependence involved both the nonspecific (on a syngeneic target cell) and the specific components of cytolysis, and was similar to the predominant Ca^{2+} dependence of cytolysis mediated by mouse T cells (refs 5–7 and P. G. and E. Smith, unpublished). Other experiments showed that, although Mg^{2+} alone could not support cytotoxicity, it tended to increase the cytotoxicity obtained in the presence of Ca^{2+}.

Lysis of antibody-coated sheep red blood cells (SRBC–Ab) by unsensitised human blood cells could occur in the absence of added cations, and was increased by the addition of Mg^{2+} more than by the addition of Ca^{2+} (Fig. 1*b*). This was found in each of five experiments, which also showed the absence of toxicity of effector cells for uncoated (control) SRBC. The predominant Mg^{2+} requirement of this system in human and also mouse[8] effector cells contrasts markedly with the cation requirements of the other cytolytic systems described here. Moreover, the cytotoxicity for SRBC–Ab observed in the absence of added cations was not entirely accounted for by cation contamination, because it was not abolished by the addition of an excess of EDTA (Table 1). Thus, lysis of SRBC–Ab by human cells has two components, one of which is predominantly Mg^{2+}-dependent and the other which cannot be blocked by EDTA and is therefore divalent cation-independent. Lysis of antibody-coated erythrocytes by normal mouse spleen cells is Mg^{2+}-dependent[8], whereas their lysis by normal mouse peritoneal cells cannot be blocked by EDTA[9]. A likely interpretation of these results is that two functionally different effector cell populations against antibody-coated erythrocytes, detected in different anatomical locations in mouse, occur in human blood. Note that we are probably dealing here with extracellular lysis, not phagocytosis, as the rate of ^{51}Cr release would be much slower in the latter case[10] and phagocytosis is inhibitable by EDTA[9].

Lysis of antibody-coated Chang human liver tumour cells (Chang–Ab) by unsensitised human blood cells was predominantly Ca^{2+}-dependent (Fig. 1*a*). This was found in each of six experiments. In some of these, it was found in addition that Ca^{2+} and Mg^{2+} together may support a somewhat higher level of cytotoxicity than Ca^{2+} alone (Table 2). This effect was linked, at least in part, to the 'spontaneous' cytotoxicity, at high ratios, of normal human cells towards uncoated Chang cells. The level of this spontaneous cytotoxicity varied from individual to individual, from very marked to minimal. Note that it was substantial only in the presence of both Ca^{2+} and Mg^{2+} (Table 2). A time course experiment (Fig. 2) also showed that it was not the absolute concentration of any divalent

Table 1 Cytotoxicity (percentage ^{51}Cr release) of human peripheral blood cells for antibody-coated SRBC, in the absence or presence of Ca^{2+} and/or Mg^{2+}, with or without EDTA

Experiment	Cytotoxic cells*	EDTA (2 mM)	Ca^{2+} (1 mM)	Mg^{2+} (1 mM)	Ca^{2+}(0.5mM)+ Mg^{2+}(0.5mM)	No added cations
1	Human blood cells	−	34	41	50	23
	None	−	6	6	6	6
	Human blood cells	+	16	15	16	16
	None	+	6	6	6	6
2	Human blood cells	−	34	39	49	27
	None	−	7	6	7	7
	Human blood cells	+	22	21	22	22
	None	+	9	9	8	9

*Normal peripheral blood cells, prepared as described in Fig. 1, tested in a 4 h ^{51}Cr release test at a ratio of 1:1 target cell, in the presence of the indicated concentration of cations and/or EDTA.

Table 2 Cytotoxicity of human peripheral blood cells for Chang cells, uncoated or coated with antibody, in the presence of Ca^{2+} and/or Mg^{2+}

		Ca^{2+}(1 mM)				Mg^{2+}(1 mM)				Ca^{2+}(1 mM)+Mg^{2+}(1 mM)			
Experiment*	Target cells	(40)†	(20)	(10)	(0)	(40)	(20)	(10)	(0)	(40)	(20)	(10)	(0)
1	Chang	4	5	3	0	2	1	2	0	23	19	17	2
	Chang–Ab	38	37	31	1	18	10	10	4	42	39	32	5
2	Chang	1	2	0	0	1	1	0	0	6	4	3	1
	Chang–Ab	22	18	11	2	7	6	7	5	26	20	14	4
3	Chang	5	2	3	0	3	2	2	3	8	8	4	3
	Chang–Ab	13	10	1	−4	−2	−3	−2	−3	24	17	8	−3
4	Chang	−3	−8	0	0	1	0	1	1	8	7	7	4

*Peripheral blood cells, prepared as described in Fig. 1, taken from four different apparently normal subjects, were tested in a 4 h ^{51}Cr release test against Chang cells, with or without rabbit anti-Chang antiserum, in the presence of the indicated concentration of cations. Cytotoxicity is expressed as percentage ^{51}Cr release minus spontaneous ^{51}Cr release in the presence of Ca^{2+} alone.

†Different ratios of effector to target cells.

cation, but the actual presence of both Ca^{2+} and Mg^{2+} which was important. Preliminary experiments indicate that the toxicity of normal human blood cells over short incubation periods is largely Ca^{2+} plus Mg^{2+}-dependent, not only in the case of Chang cells, but also for other human tumour cell lines.

We found that different types of human cell-mediated cytolysis have different cation requirements. Lysis of PHA blasts mediated by T cells requires Ca^{2+}, lysis of SRBC–Ab mediated by non-T cells preferentially requires Mg^{2+} or no cations, lysis of Chang–Ab mediated by non-T cells requires Ca^{2+}, and the anti-Chang spontaneous cytotoxicity requires both Ca^{2+} and Mg^{2+}. Note that these differences in cation requirements are relative. For instance, in every system requiring either Ca^{2+} or Mg^{2+}, the 'non-required' cation, poorly effective by itself, tends to increase the cytotoxicity obtained in the presence of the 'required' cation. The anti-Chang system may represent an extreme expression of the same phenomenon. Also, the results suggest that the differences in cation requirements are not exclusively linked to the nature of

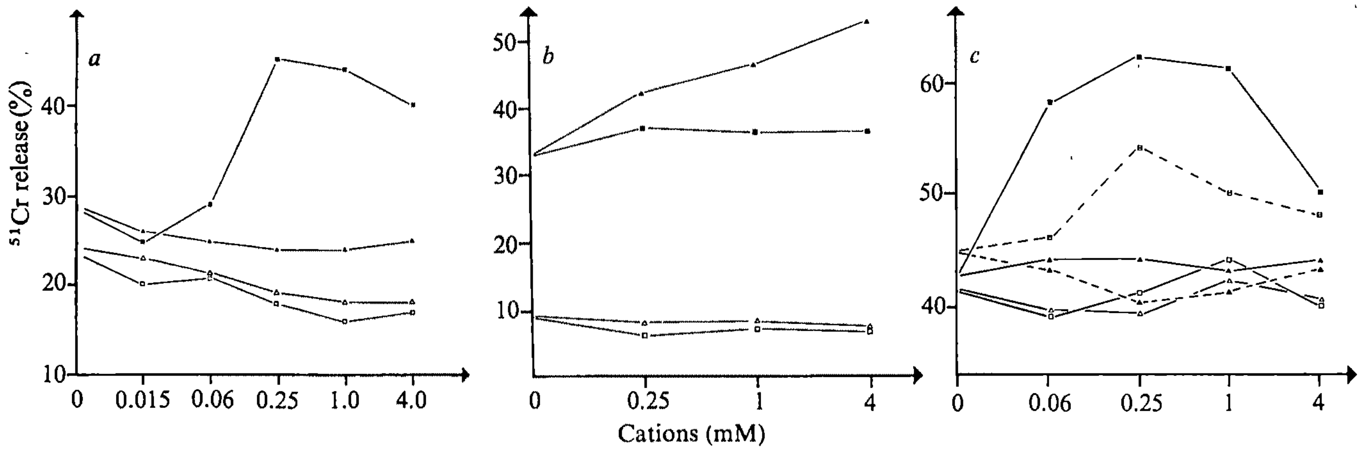

Fig. 1 Cytotoxicity (expressed as ^{51}Cr release) of human cells for various types of target cells, in the presence of graded concentrations of Ca^{2+} (□, ■) or Mg^{2+} (△, ▲). Cytotoxicity was measured using a 4 h ^{51}Cr release test, after the addition of known amounts of Ca^{2+} and/or Mg^{2+} to systems initially as free as possible of divalent cations. Three measures were taken to reduce unwanted cation contamination. First, we used only disposable plastic material, not washed glassware. Second, both cytotoxic and target cell populations were pretreated with Versene (1.5 mM EDTA solution). Third, the medium used for all subsequent steps, including the cytotoxicity test, was a modified Eagle's medium specially prepared without CaCl$_2$ and MgCl$_2$ (ICRFL) plus 1% heat-inactivated foetal calf serum (FCS) dialysed for 48 h against double-distilled water. This percentage of FCS was chosen in preliminary experiments as a compromise between lower percentages (detrimental to lysis mediated by T cells) and higher percentages (blocking lysis mediated by non-T cells of antibody-coated erythrocytes). As it is, this medium could be used for all types of cell-mediated cytolysis described in this report. Its contamination by divalent cations, repeatedly checked by atomic absorption spectrophotometry, averaged 0.036 mM and 0.010 mM for Ca^{2+} and Mg^{2+}, respectively. Human peripheral blood cells, sensitised or not, were incubated in a Versene solution for 30 min at 37 °C, washed twice, adjusted to the required concentration and distributed in V-shaped wells of IS-MVC-96 Linbro microplates. Each well also received target cells (5×10^3 PHA blasts or 10^6 SRBC or 10^4 Chang cells, labelled overnight with ^{51}Cr as previously described[15], washed once with Versene and twice with medium), antiserum when required (rabbit anti-Chang or rabbit anti-SRBC antisera, at a final concentration of 1:10,000) and solutions of CaCl$_2$ or MgCl$_2$ to a total volume of 0.02 ml. The microplates were centrifuged (280g, 2 min) to increase the sensitivity of the cytotoxicity test[15,16], incubated for 4 h at 37° C in a humidified CO$_2$ incubator and centrifuged again. Volumes (0.1 ml) of supernatants were sampled with an Eppendorf micropipette, their radioactivity measured and expressed as percentage total radioactivity per well. In this type of assay, an experimental difference of 5% or more is statistically significant ($P = 0.05$). *a*, Cytotoxicity of normal human cells obtained by Ficoll–Triosil fractionation of defibrinated blood, at a ratio of 10:1 Chang–Ab (■, ▲). □, △, Spontaneous release by Chang–Ab alone. *b*, Cytotoxicity of normal human cells obtained by Ficoll–Triosil fractionation of heparinised blood, at a ratio of 1:1 SRBC–Ab (■, ▲). □, △, Spontaneous release by SRBC–Ab alone. *c*, Cytotoxicity of sensitised cells for PHA blasts. Normal human cells were obtained by Ficoll–Triosil fractionation of defibrinated blood of subjects A and B. A anti-B and B anti-A sensitised cells were obtained after 6 d of coincubation at 37 °C in a 10% CO$_2$ atmosphere of 2×10^6 responding human cells and 2×10^6 irradiated (2,000 rad) stimulating human cells, in 2 ml of modified Eagle's medium plus glutamine and 10% human serum, per well of FB-16-24-TC Linbro plates. Either A or B target PHA blasts were obtained by incubating normal cells for 3 d in the presence of PHA at a concentration of 1:100. For the cytotoxicity test, the effector–target cell ratio was 15:1. If target cells are PHA blasts obtained from A cells; ■, ▲, effect of B anti-A; --□--, --△--, effect of A anti-B sensitised cells; □, △, spontaneous release by PHA blasts alone.

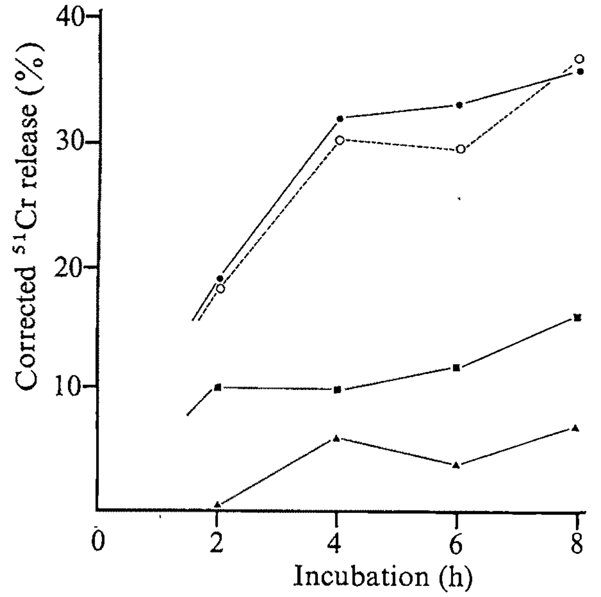

Fig. 2 Cytotoxicity of normal human peripheral blood cells for Chang cells, expressed as corrected ^{51}Cr release (^{51}Cr release minus spontaneous ^{51}Cr release by target cells alone), at a ratio of 100 effector cells to 1 target cell, as a function of time and of the presence of either 1 mM Ca^{2+} (■), or 1 mM Mg^{2+} (▲), or 1 mM Ca^{2+} plus 1 mM Mg^{2+} (●), or 0.5 mM Ca^{2+} plus 0.5 mM Mg^{2+} (○).

the target cells. These findings have implications for both the mechanism of lysis and the nature and fractionation of cytotoxic cells. As for the mechanism of lysis, the difference in cation requirements described here do not seem to apply to the recognition step of cytolysis (compare with refs 11–14) and thus may well reflect differences between the actual post recognition lytic mechanisms of various systems (P. G. and E. Smith, unpublished); and the results obtained in the anti SRBC–Ab system reinforce our previous conclusion[8] against a conventional, extracellular Ca^{2+}-dependent, 'stimulus–secretion' mechanism in this type of cytolysis. The potential of a functional fractionation of cytotoxic cells on the basis of their cation requirements is demonstrated by the specification of the individual characteristics of the cytotoxic activity of normal human blood cells against antibody-coated erythrocytes from other cytolytic systems, and its further dissection into two functional components. Furthermore, when tumour cells are used as targets, our results suggest that Mg^{2+}-free conditions may depress the cytotoxicity of normal control cell populations more than specific anti-tumour cytotoxicity, which would be an obvious advantage in anti-tumour research in man.

We thank Miss L. Strong for assistance, Dr I. C. M. MacLennan for Chang cells and rabbit anti-Chang antiserum and the MRC, Imperial Cancer Research Fund and INSERM for support.

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Received March 4; accepted April 21, 1975.

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Radiation response of C3H fibrosarcoma enhanced in mice stimulated by *Corynebacterium parvum*

TREATMENT of the laboratory mouse with *Corynebacterium parvum* can potentiate efficacy of host reaction against tumours[1-4]. This has been reflected in the proportion of mice whose tumours: (1) grew more slowly; (2) regressed partially before continuing growth, and (3) regressed completely, either temporarily or permanently. Furthermore, in some mouse tumour systems chemotherapy is more effective when hosts are stimulated with *C. parvum*. We have now found that administration of *C. parvum* before local irradiation of 8-mm diameter fibrosarcoma isotransplants increases the frequency of cure, strikingly so after low radiation doses.

A C3H mouse fibrosarcoma (induced by methylcholanthrene) was used as sixth generation isotransplants growing in the right legs of C3Hf/Sed mice (bred and maintained in our colony which is pathogen-free and carries a defined flora)[8]. The fibrosarcoma has been shown to be immunogenic by quantitative cell transplantation studies in normal, tumour-immunised, and immunosuppressed recipients[8]. Treatment with *C. parvum* began 7–9 d after transplantation when the tumours were 5 mm in diameter. We injected 0.35 mg intravenously (0.4 ml) or intralesionally (0.1 ml of the suspension was deposited along six needle tracks around and through the tumour). When the tumours reached 8 mm in diameter, ^{137}Cs radiation was given as a single dose at 950 rad min^{-1}. Radiation technique was based on a 3-cm diameter treatment portal so that only the tumour and the affected limb were irradiated. For irradiation, the mice were taped to a restraining plate in a 250-ml chamber, sealed and ventilated with 2 l of sterile air per min. Results have been tabulated as the proportion of mice free of tumour 80 d after treatment.

Table 1 shows that up to 60% of mice were cured permanently of their 5-mm diameter tumour by *C. parvum* alone. In successfully treated mice, tumour growth did not differ from that in control mice for 5–10 d after injection of *C. parvum*; regression then commenced and was usually complete within another 10 d. Regression was more likely if *C. parvum* was administered both intralesionally and intravenously than by either route alone; the sequence of injection routes did not seem important. The remaining data demonstrate a significant increase in tumour control after local irradiation in mice pretreated with *C. parvum*. The efficacy of *C. parvum* was not demonstrably greater when it was administered intravenously and intralesionally when the tumour was also subjected to local irradiation. The increased efficacy of administering *C. parvum* and local irradiation was most impressive for small doses of radiation. The difference in frequency of tumour destruction between control and *C. parvum* treated animals whose tumours received 800 or 1,500 rad was significant at $P \ll 0.01$.

The difference in the probability of survival between the mice given radiation alone and those given *C. parvum* and radiation was 0.68 with low doses (800–1,500 rad) and 0.11 with higher doses (2,250–3,750 rad); the difference between these two values is significant ($P=0.007$). (χ^2 test for heterogeneity). This finding that *C. parvum* potentiates the efficacy of radiation administered at low but not at high doses, in this tumour system, emphasises the need for information about the role of radiation-induced changes in tumours and in adjacent normal tissue if the efficacy of the host's attempt to reject a

Table 1 Proportion of mice free of tumour 80 d after irradiation

C. parvum	0	200	400	800	1,500	2,250	3,000	3,750
None	0/20		0/5	0/10	2/10	2/5	4/9	14/15
Intravenous								
5-mm tumour	10/29	8/10	8/10	10/14	13/15	9/15	9/10	11/13
Intralesional								
5-mm tumour	2/10			0/4	1/5	4/5	4/5	4/5
Intravenous								
5-mm tumour	11/20	8/10	10/10	12/15	5/5	2/4	4/5	4/4
Intralesional								
8-mm tumour								
Intralesional								
5-mm tumour	6/10			3/4	4/5	3/5	4/4	4/5
Intravenous								
8-mm tumour								

Figures show radiation dose–tumour control response data for 8-mm diameter fibrosarcoma (isotransplants in right leg) alone or combined with *C. parvum*.

tumour graft is to be modified. Although the precise mechanism of action of *C. parvum* is not known, the observation of such a powerful adjuvant effect with local irradiation may have potential interest for radiation therapy.

This work was supported in part by a grant from the US Public Health Service. Burroughs Wellcome provided *C. parvum*. Statistical analysis of the data was performed by Dr K. Rothman, Harvard School of Public Health.

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Received February 12; accepted April 21, 1975.

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Drug-induced phase change in bilayer as possible mode of action of membrane expanding drugs

MANY small molecules modulate membrane functions. Substances such as neurotransmitters interact directly with specific protein receptor sites, whereas others such as anaesthetics, interact with membrane lipids or with hydrophobic regions of membrane. This is consistent with a correlation of their activity with their lipid solubility and lipid–water partition coefficient[1]. Several lipid-soluble drugs are antihaemolytic[2], which correlates not only with their pharmacological activity but also with their ability to expand the lipid bilayer of a biomembrane[3]. Although expansion of bilayers implies a drug-induced reorganisation of lipids, the mechanism involved remains to be established. We now have evidence that the lipid-soluble drugs may induce a transition of lipid acyl chains from organised gel to randomised liquid crystalline phase. Such drug-induced changes in the lipid phase in a bilayer may affect the function of various membrane-bound proteins[4].

The effect of drugs on phase transition behaviour of mechanically dispersed dipalmitoyl lecithin (DPL) liposomes was studied by differential scanning calorimetry. Samples for scanning contained 75 mM DPL, 50 mM KCl, 5 mM Tris-HCl and the appropriate concentration of drug (0.1–10 mM) at *p*H 7.4. After mixing, the samples were allowed to equilibrate for 2–3 d at 4 °C before they were scanned on DSC-1B (Perkin Elmer) in sealed sample pans. For all runs a sensitivity of 1 mcalorie s^{-1} was used with a scanning rate of 1.25 K min^{-1} between 295 and 320 K. The enthalpies of transition were calculated from the areas under the curves, which were determined by weighing the traces of the transition profiles.

The transition profile for DPC dispersion without any drug showed a transition temperature at 314.6 K with enthalpy of the transition +8.9 kcalorie mol^{-1}, and the width and half-height width for the transition as 1.6 K and 0.60 K respectively. In the liposomes which contained a drug,

Table 1 Concentrations of various drugs which double the width at half-height of the transition profile

Drug	Conc. (mM)	Type of transition profile*
Alkanols		
n-Pentanol	23	A
n-Hexanol	5.1	A
n-Heptanol	2.5	A
n-Octanol	1.14	A
Local anaesthetics		
Procaine	17	A
Nesacaine	15	A
Benzocaine	3.5	A
Butacaine	3.0	A
Tetracaine	3.3	A
Dibucaine	1.8	A/B
Xylocaine	6.7	A
Marcaine	2.4	A
Uncouplers		
TTMB	3.3	B
CCP	7.5	B
Cl-CCP	5.3	B
FCCP	4.4	B
Tranquillisers		
Prochloroperazine	0.7	A
Trifluoroperazine	1.6	A
Trimeprazine	5.2	A
Chlorpromazine	4.5	A
Inhalation anaesthetics		
Diethyl ether	85	C
Chloroform	10	C
Halothane	3.1	A/C
Fluroxane	4.5	C
Enflurane	25.5	C

* TTMB (3,4,5,6-tetrachloro-2-trifluoromethylbenzimidazole); FCCP, (carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone); CCP, (carbonylcyanide phenylhydrazone); Cl-CCP, (*m*-chloro-CCP). For type *B* profiles, the concentration given reduced the area under the main peak by 50%. For type *C* profiles, the concentration given shifted the peak at half-height by 0.6 K. Some drugs, like dibucaine and halothane, showed two types of profile. The first at low concentrations and the second at higher concentrations.

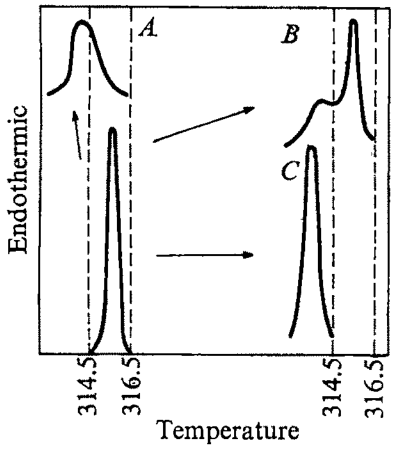
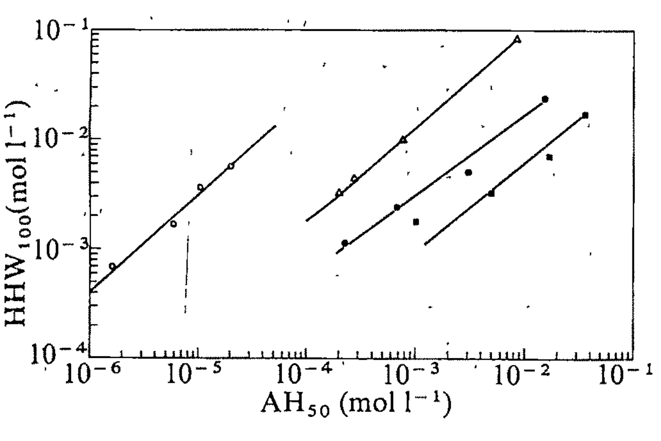


Fig. 1 Typical DSC transition profiles for DPL liposomes doped with various drugs. Three types of profiles have been observed for the drugs shown in Table 1. In type *A*, an asymmetrical broadening of the main peak is observed. This broadening is accompanied by an increase in the half-height width and a decrease in T_c. In type *B* profiles, the area of the main peak decreases, and the area of the shoulder at lower temperatures increases with increasing drug concentration. T_c for the main peak seems to remain unchanged, whereas T_c for the shoulder decreases at higher drug concentrations. In type *C* profiles the main peak shifts to lower temperatures at successively higher drug concentrations. No broadening of the peak is observed. The total area under the curves (enthalpy of transition) seems to remain constant in all these profiles. In the temperature range for transition, several phases may coexist within the plane of the bilayer.

the transition profile was characteristic for the type of drug. In general, three distinct types of transition profile (Fig. 1) were obtained for the drugs examined (Table 1). Thus all uncouplers of oxidative phosphorylation showed type *B*, local anaesthetics showed type *A*, and inhalation anaesthetics showed type *C* transition profiles. For all drugs, a broadening of the transition profile accompanied a lowering in the phase transition temperature (T_c) of DPL.

For most of these doped bilayers, however, there was little or no change in the enthalpy of transition. The effect of various drugs on half-height width (type *A*), relative area (type *B*) or shift (type *C*) of the main transition peak was linearly concentration-dependent and did not seem to show a saturation behaviour in the range (up to 10 mM for most drugs) we examined.

Fig. 2 The correlation between the concentrations of drugs that increase the half-height width by 100% (HHW_{100}; data from Table 1) and concentrations of the same drugs which protect human erythrocytes by 50% against haemolysis (AH_{50}; data from ref. 2). For inhalation anaesthetics the abscissa is AH_8 (8% protection; data from ref. 3) and ordinate is the concentration at which the transition peak shifts by 0.6 K at half height. ●, *n*-Alkanols; ■, local anaesthetics; ○, phenothiazine tranquillisers; △, inhalent anaesthetics.



These effects on transition profiles therefore relate to the fraction of the bilayer modified by the drug. Regardless of the mechanism of incorporation of the drugs in bilayers, their effects on transition profile seem to depend on their concentration in the liposome. Thus the effects on transition profiles may be correlated with other concentration-dependent effects on membrane, such as antihaemolytic activity. As Fig, 2 shows for a series of drugs, the concentration that induces a certain effect on the liposome bilayer (Table 1) is related to the corresponding concentrations for 50% or 8% antihaemolysis of human red cells[2]. Correlation for each drug type is good. Variation between the drug types is quite large. All the lines seem, however, to be parallel. This variation may imply a difference in the partition coefficient for these drug types in DPL liposomes and human erythrocytes. Only dibucaine, which has a structure different from other anaesthetics, does not fall on the line for local anaesthetics.

The transition from gel to liquid crystalline phase in a phospholipid bilayer can be described as a highly cooperative transition from order to disorder, involving a lateral expansion and a decrease in thickness and density[7,8]. Within the transition range (Fig. 1), two-dimensional domains of fluid and gel phase coexist. Below and above this range, the membrane exists in gel and fluid phases respectively. The data presented here show that addition of lipid-soluble drugs not only lowers the transition temperature but also enlarges the temperature range in which two-dimensional domains of fluid and gel phases coexist. Therefore, in the presence of drugs at a given temperature a greater fraction of membrane is in the fluid phase. These results support the conclusion that anaesthetics and related lipid-soluble drugs cause an increase in fluidity, disorder and mobility of the lipid bilayer [8–10] which can modulate the function of membrane proteins[4,11].

These observations correlate the pharmacological activity and membrane expanding ability of various drugs to their ability to lower the gel to liquid crystalline transition temperature in lecithin bilayers (Fig. 2). A rationale for this correlation can be seen in the suggestion that within an optimum temperature range the domains of gel and liquid crystalline state may coexist in the bilayer of biomembranes[12]. A drug-induced decrease in the transition temperature would imply that in biomembranes small molecules can induce effects similar to those elicited by increasing temperature in a critical range. This hypothesis is also consistent with the observation that the volume expansion of membrane is about ten times the bulk volume of the drug[2] that induces it. Drugs may also affect the proportion of lipid in the two or more phases which coexist within a bilayer. Consequently, a drug-induced increase in membrane fluidity would result largely from a simultaneous increase in the fluid and a decrease in the gel region.

This work was supported by a grant from the Delaware Institute for Medical Research. We benefited considerably from discussions with Drs Lynda and Karl Koehler, University of North Carolina.

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Received March 19; revised April 9, 1975

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Voltage distribution across nerve membranes

EXPERIMENTS on nerve membranes and model systems (such as black lipid films modified by excitability-inducing material) often show conductance parameters which seem to be closely related to Maxwell–Boltzmann statistics[1]. There are, however, some difficulties when trying to fit simple Maxwell–Boltzmann factors to experiments. One often has to introduce an effective charge that is not an integer times the electronic charge and often the effective charge changes with voltage. We show here that the effective charge and its nonlinearity may be a consequence of the voltage distribution across the membrane. This implies that the so-called gating particles[2] may be univalent charges—for example, electrons (I.L. and M.S., unpublished).

By assuming that the active areas in a membrane are surrounded by polar regions that are polarisable, that is, the dipole moments change with electric field we show that the voltage drop (V_a) over the portion of membrane inside the polar regions can be larger than the total membrane voltage V.

The exact calculation of the behaviour of these polarisable dipole layers in the presence of a potential difference between the inside and outside of the membrane is a rather difficult task involving the interplay between electrical and molecular forces, and the nature and distribution of the dipoles. Furthermore, if the dipoles are located mainly at the conducting channels our problem is two or three-dimensional and not one-dimensional as in ordinary calculations of membrane potential distributions.

A simple qualitative illustration is given in Fig. 1. The voltage distribution along an ionic channel is shown for different voltage differences, V, across the membrane. The presence of polarisable dipole layers is seen to change the voltage across the active region, V_a, more than the change in $V-V_o$ (Fig. 1), where V_o is the voltage on the outside of the membrane. This is caused by a change in the voltage drop across the dipole layers. The voltage drop across a dipole layer is, in principle, given by the number of dipoles, their density and their moment, that is the distance (in the x direction) between the positive and negative charge. For polarisable dipoles this distance is determined by a balance between electrical and mechanical (molecular) forces working on the charges in the dipoles (Fig. 1).

In a simple one-dimensional model the dipoles are regarded as a positive and negative sheet charge separated by a distance, x. We obtain

$$\begin{aligned} V_a &= (V-V_o)[1-(2\varepsilon_a\sigma^2/\varepsilon^2_d d\cdot K)] \\ &= (V-V_0)/(1-\gamma) \end{aligned} \qquad (1)$$

for the special case of identical dipole layers at the outside and inside of the membrane, neglecting any extranegative surface charge and any change in voltage drop across the Gouy–Chapman layers. σ is the density of positive (and negative) charge, ε_a and d the dielectric permittivity and thickness of the active region, respectively (Fig. 1). ε_d is the dielectric permittivity of the material in the dipole layer, K is a force constant (N/m^3) describing the mechanical (molecular) forces acting on the charges in the dipoles. Equation (1) was derived assuming $x \ll d$. The change in equilibrium distance, x, between the positive and negative charges in the dipoles associated with equation (1) is

$$\Delta x = \pm(\varepsilon_d\,\gamma/2\sigma(1-\gamma))\cdot(V-V_o) \qquad (2)$$

($\gamma < 1$ is a necessary condition for a stable system with minimum

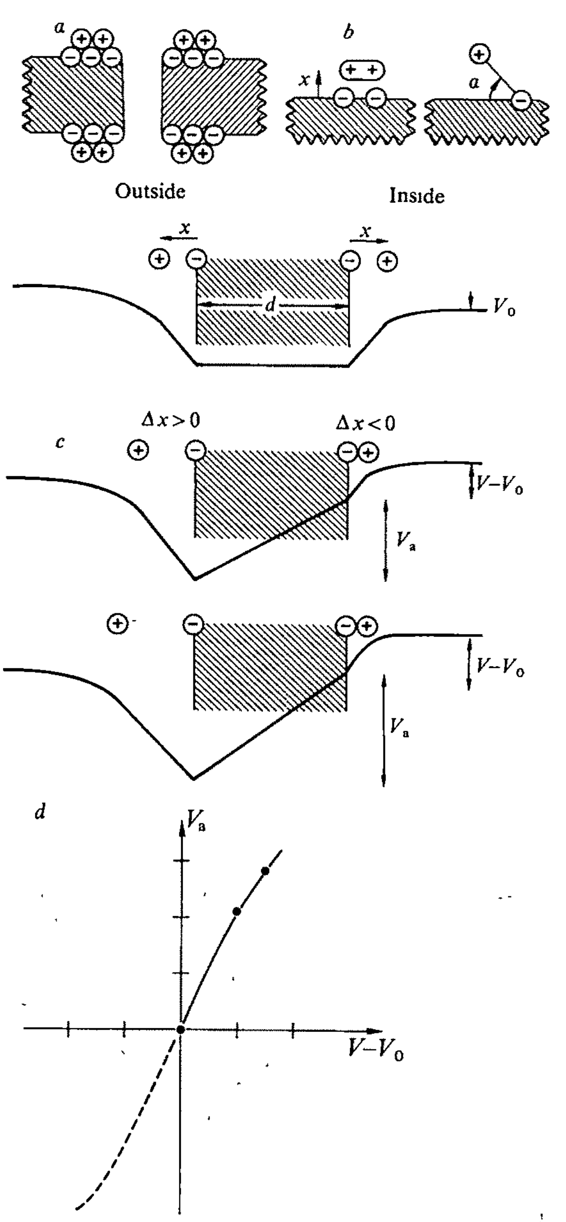

Fig. 1 Schematic illustration of the influence of polarisable dipoles on the voltage distribution across an ionic channel. *a*, Channel with dipoles at the outside and inside of the membrane wall. More negative than positive charges are shown to illustrate the presence of fixed negative charges at the membrane wall. *b*, Two hypothetical types of dipoles; positive charge (in this case a divalent ion) adsorbed to the negative charge at the channel and a permanent dipole at the channel. In both cases it is assumed that the distance between the positive charge and the membrane wall depends on the electric field perpendicular to the wall. The value of x or α is determined by a balance between electrical forces and molecular forces acting on the charges. In our simple model the negative charge is assumed to be fixed. *c*, Voltage distribution along an ionic channel for different voltage differences V between the outside and the inside of the membrane. *d* Is the length of a channel inside the polar region. The change in equilibrium position between the positive and negative charge in the dipole is made very large for illustrative purposes. In reality $|\Delta x|$ will be small compared with the membrane thickness. The last picture illustrates the fact that when the charges get close to each other very small changes in x can be expected because of large repulsive forces. It is seen that $V_a > V-V_o$ because of the change in the voltage drop across the dipole layer. Voltage drops across the Gouy–Chapman layers indicated in the drawing are assumed to be approximately constant. *d*, V_a versus $V-V_o$ obtained for the model presented in (*c*). Dots correspond to the different drawings in (*c*). Negative part of the curve can be constructed in a similar way. Because of the properties of the dipole layers on the outside and inside (one may even be missing), V_a as a function of $V-V_o$ may be asymmetric around $V-V_o=0$.

total energy.) Experiments on nerve axons and model systems indicate that the gating particle has (or the gating process behaves as) an apparent valence which (1) varies with V and (2) has values varying between say 1 and 5. It is evident that an explanation may be found in the voltage distribution across the membrane instead, as equation (1) implies that a particle with charge q will behave like a particle with charge $q/(1-\gamma)$ related to the applied voltage V.

It is seen that dipoles with large enough polarisability (small enough K) will give a γ of sufficient magnitude to account for point (2) above. $\gamma=0.5$, which corresponds to $V_a=2(V-V_0)$, requires for example $\Delta x=\pm 0.0025(V-V_0)$ Å for $V-V_0$ in mV if $\sigma=10^{18}$ electronic charges per m^2 and $\varepsilon_a=\varepsilon_d=10\varepsilon_0$. Small (field-dependent) displacements of the charges in the dipole layers thus give a considerable dependence of V_a on $V-V_0$.

Point (1) above, which is equivalent to a nonlinear dependence of V_a on $V-V_0$, is easy to understand in the present model. When the positive and negative charges are pushed close to each other the intermolecular repulsion forces (as a result of overlap of electronic orbitals, for example) are increased rapidly giving rise to a smaller Δx for a given change in $V-V_0$ (to a larger force constant K in equation (1)). The result will be a nonlinear V_a versus $V-V_0$ (Fig. 1).

Thus, to obtain a nonlinear relationship between the active voltage V_a and the applied voltage V with slope >1 we need dipoles with a suitable polarisability close to the ionic channels. We are not restricted to the situation shown in Fig. 1. Dipoles may be present only on one side of the membrane. It is also possible that dipole layers with opposite polarity may be present on the same side of the membrane. The necessary dipoles may be an integral part of the channel molecules or their closest surroundings. These dipoles may also consist of fixed negative and adsorbed positive charges. These two possibilities agree with other theories and experiments on nerve membranes and model membrane systems[3,4].

A necessary consequence of the present model is that the voltage distribution across the membrane wall (in the region of an ionic channel) is largely determined by the dipole layers. A general property of the model is that the larger the polarisability is, the smaller K is, the larger will be the parameter γ.

The most important conclusion from the above is that the nonlinearity of nerve membranes and model systems may be explained by the relationship of the voltage drop across the active parts of the membrane and the externally applied voltage. It is not necessarily explained by gating particles with a large electronic charge or by the cooperative movement of a number of charged entities. In a recently proposed electronic model for nerve membrane excitability we used this result (I.L. and M.S., unpublished). The Hodgkin–Huxley parameters were satisfactorily reproduced using a slightly nonlinear V_a against V relationship with $\gamma_{max} \approx 0.5$. A more detailed calculation on the presented model will be given elsewhere.

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Received November 28, 1974, accepted April 21, 1975.

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Effects of oestrogen and bromocryptine on *in vivo* secretion and mitosis in prolactin cells

THE response of the rat pituitary gland to oestrogen includes increased mitotic and secretory activity affecting several of the cell types in the anterior lobe but leading in particular to proliferation of prolactin cells[1]. Bromocryptine inhibits release of prolactin[2,3] and also inhibits DNA synthesis in the oestrogen-stimulated pituitary gland[4]. In studying the pituitary response to oestrogen during a prolonged period of bromocryptine inhibition, we have observed a close relationship between effects on serum prolactin levels and on pituitary mitotic activity. Our findings lend support to the idea that secretory behaviour influences mitotic activity in prolactin cells[4].

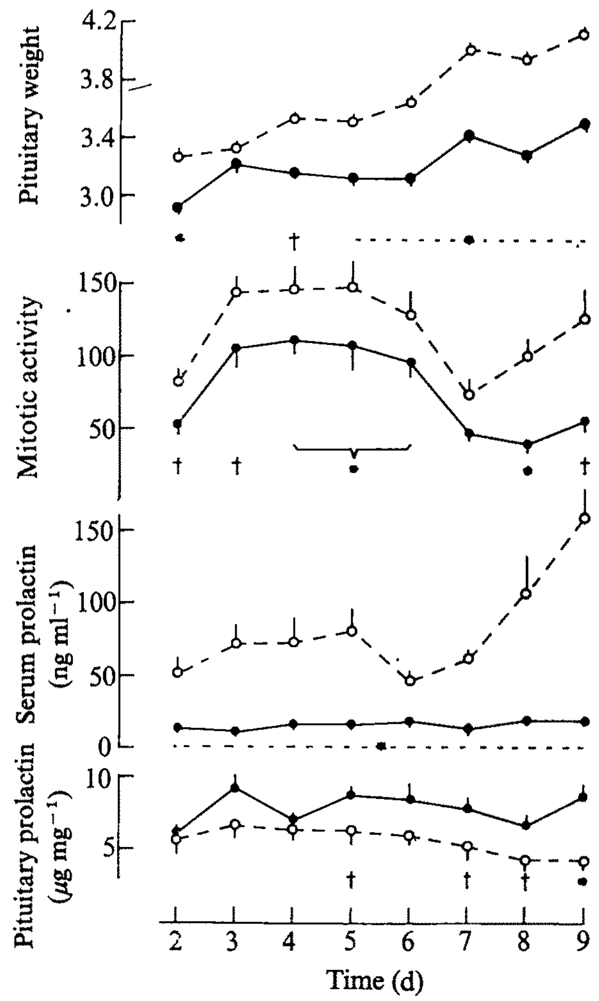

Fig. 1 Effects on male rats of a single dose of DESD (12 mg subcutaneously given on day 0) with (●) and without (○) bromocryptine (0.3 mg per 100 g body weight subcutaneously, given daily from day −1 to +8). Mean values (±s e.) in groups of seven rats for pituitary weight (mg per 100 g body weight) mitotic activity (mitotic figures mm^{-2} pituitary section × pituitary weight) serum prolactin and pituitary prolactin concentration. Significance of difference (Student's t test): *, $P<0.01$; †, $P<0.05$.

Male Sprague–Dawley rats (3–4 months old), maintained in a light- and temperature-controlled room on tap water and a pellet diet *ad libitum*, were given a single subcutaneous dose of 12 mg diethylstilboestrol dipropionate (DESD) in oil on day 0. On day −1, and daily thereafter, the rats were given either bromocryptine (0.3 mg per 100 g body weight) or vehicle subcutaneously at 1600. On each of days 2–9, a test group (given DESD and bromocryptine) and a control group (given DESD and vehicle), seven rats each, were given colchicine in water (0.1 mg per 100 g body weight) intraperitoneally at 0900. Rats were anaesthetised with chloroform 1–1.5 h later and bled by section of the neck arteries until dead. Blood was kept for radioimmunoassay of prolactin and growth hormone. Pituitary glands were weighed and halved sagittally; one half was used for radioimmunoassay for prolactin and growth hormone, the other was fixed in Bouin–Hollande and 5 μm sections cut and stained with haematoxylin and eosin. Two to

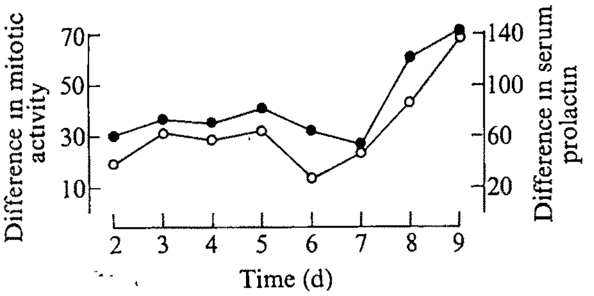

Fig. 2 Differences on each day in mean pituitary mitotic activity (●) and in mean serum prolactin (○) between rats given DESD and bromocryptine, and rats given DESD and vehicle.

three sections of each hemi-pituitary gland (50,000–70,000 cells) were examined for cells in mitosis.

The pituitary response to the single dose of DESD (Fig. 1) was similar to that described previously[4]—increasing pituitary weight and serum prolactin concentrations, and a later rise in serum growth hormone. The first 'wave' of mitotic activity subsided partially by day 7 and the subsequent rise was accompanied by rising serum prolactin and growth hormone. Bromocryptine inhibited the oestrogen-induced gain in pituitary weight, markedly reduced serum prolactin levels (Fig. 1) and had little effect on serum growth hormone. Pituitary prolactin concentrations were raised by bromocryptine whereas growth hormone showed no consistent change. Pituitary mitotic counts were reduced by bromocryptine on each of days 2–9, although the differences between test and control groups varied considerably. These differences were compared with those in serum prolactin and, for this purpose, the mitotic activity in each pituitary gland was derived by multiplying mitotic index (number of cells in mitosis per mm^2 pituitary section) by pituitary weight. The differences on each day in mean mitotic activity and in mean serum prolactin followed a similar pattern (Fig. 2) and were significantly correlated (Fig. 3).

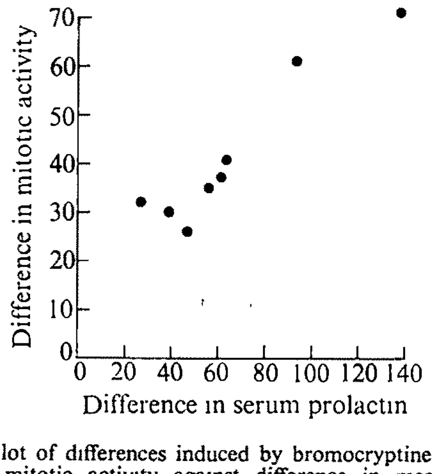

Fig. 3 Plot of differences induced by bromocryptine in mean pituitary mitotic activity against difference in mean serum prolactin on each of days 2–9. $r = +0.94$; $P < 0.001$.

Our findings suggest that bromocryptine inhibited proliferation of prolactin cells, thus accounting for the smaller gain in pituitary weight and the lower mitotic activity when compared with the effects of oestrogen alone. Functionally, the prolactin cells under the influence of bromocryptine and oestrogen maintained low serum prolactin levels and high intracellular concentrations. The oestrogenised pituitary glands, without bromocryptine, stored less and released more prolactin. The close correlation between the inhibitory effects of bromocryptine on mitotic activity and on serum prolactin may be interpreted in various ways. A direct effect of bromocryptine on DNA synthesis is a possibility but, to account for our findings, such an effect would have to be restricted to the prolactin cells, enabling them to continue to maintain intracellular stores of prolactin and low but measurable serum prolactin levels. In inhibiting release of prolactin, bromocryptine could be acting by lowering pituitary cyclic AMP levels, this substance having been shown to stimulate prolactin release[5,6]. Altered cyclic AMP levels may then influence DNA synthesis but, from studies on other tissues[7,8], a rise rather than a fall in cyclic AMP would be expected to lower pituitary DNA synthesis. Hypothalamic factors influenced by bromocryptine and oestrogen could also be involved in control mechanisms acting on mitotic[9] as well as secretory activity[10] in the pituitary gland. The two agents could be specific antagonists at either pituitary or hypothalamic levels. As the pituitary glands in the rats given bromocryptine maintained the higher prolactin concentrations, however, the effect of oestrogen on prolactin production did not seem to be inhibited by bromocryptine. There remains the possibility that bromocryptine acts exclusively by inhibiting prolactin release and that this in turn leads to inhibition of mitosis.

We reported previously[4] that bromocryptine inhibition of DNA synthesis in oestrogenised pituitary glands followed the effects on prolactin release and suggested that the bromocryptine-induced rise in intracellular prolactin concentration could be a factor leading to inhibition of DNA synthesis. Our findings may mean that prolactin cells cannot divide under the influence of oestrogen unless they are free to release prolactin. An intracellular negative feedback may exist therefore between intracellular concentrations of the hormone produced and DNA synthesis. Such a mechanism would serve to inhibit cell division in cells containing adequate stores of their product and to trigger DNA synthesis when stores were depleted as a result of release exceeding production.

This investigation was supported by the National Health and Medical Research Council, Canberra and the Queensland Cancer Fund. Mrs Carmel Ryan, Mrs Dorothy Lucas and Miss Margaret Lynch provided assistance. We thank the National Institute of Arthritis, Metabolic and Digestive Disease, Rat Pituitary Hormone Distribution Program for providing materials for radioimmunoassay of rat prolactin and growth hormone. Bromocryptine was supplied by Professor E. Flückiger and Dr H. Friedli (Sandoz Ltd, Basle).

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Metronidazole (Flagyl): iron catalysed reaction with sulphydryl groups and tumour radiosensitisation

THE possibility of using hypoxic cell radiosensitising drugs as adjuncts in the radiotherapy of some forms of cancer has received renewed attention[1-4]. In particular administration of metronidazole (Flagyl, May and Baker Ltd) to tumour bearing mice before exposure to X rays, has been found to reduce the radiation dose required to control 50% of the tumours by a factor of 1.2–1.3 (refs 5 and 6). Preliminary clinical trials are now in progress[7]. The drug was originally chosen for study because of its constituent nitro group (which suggested it was a strong radical oxidant) and its extremely favourable pharmacological and toxicological properties[8]. Previously the activity of a large number of radiosensitisers had been attributed to their ability to react with intracellular sulphydryl compounds[9,10]. The ability of the cell to repair radiation-induced free radical lesions by hydrogen[11] or electron donation[12] would thus be lowered. Measurements[13] of anoxic solutions over a period of 2 h did not, however, show any appreciable reaction between metronidazole (8×10^{-4} M) and the sulphydryl groups of cysteamine (8×10^{-4} M) or dithiothreitol (4×10^{-4} M). Recent associated pharmacological studies with rat intestinal contents have prompted us to re-examine the reaction of metronidazole with sulphydryl compounds and to investigate the possibility of catalysis by ferrous ions. We now report experiments which show that the drug reacts with an iron–cysteine complex over time scales of the order of a second. A drug–iron–cysteine complex is formed which is subsequently reduced over a period of minutes.

Solutions containing cysteine hydrochloride (0.01–0.1 M, titrated to pH $= 7.5\pm0.1$ by addition of KOH), and metronidazole (10^{-3} M) were purged with nitrogen in the absence and presence of ferrous sulphate (0.2–1 mM). Aliquots (0.5 ml) were removed at intervals and the concentration of metronidazole determined spectrophotometrically after extraction into ethyl acetate (5 ml). Unlike its reduction products the drug has an absorption maximum at 320 nm (max $\varepsilon = 9{,}310$ M^{-1} cm^{-1}) characteristic of its nitro substituent (Fig. 1*a*). In the absence of ferrous ions, negligible removal of metronidazole occurred over a period of 2 h. In solutions containing the metal ion, however, levels of metronidazole decreased exponentially with half lives ($t_{\frac{1}{2}}$) ranging from 6 to 140 min, and varying inversely with both ferrous ion and cysteine (RS$^-$) concentration (Fig. 1). Following preparation the solutions rapidly developed a purple colour, which later decayed during the experiment. Information concerning the rate of formation of the colour was subsequently obtained using a stopped-flow apparatus (Nortech Ltd). On mixing equal volumes of nitrogen purged solutions, one containing metronidazole (RNO$_2$, 2×10^{-2} or 5×10^{-2} M), the other cysteine hydrochloride (0.1 M) and ferrous sulphate (4×10^{-3} M, pH $= 7.5\pm0.1$) the light transmitted at 500 nm decreased exponentially (Fig. 2). The decay half life varied inversely with metronidazole concentration: RNO$_2$ $= 10^{-2}$ M and 2.5×10^{-2} M $t_{\frac{1}{2}} = 1.3$ and 2.3 s respectively. The above experiments were also undertaken with oxygen saturated solutions. Negligible removal of metronidazole occurred although the solutions again became coloured. A similar colour has been previously observed in oxygenated solutions in the absence of metronidazole and can be attributed[14,15] to a cysteine–iron complex (II) formed according to:

$$\mathrm{Fe^{2+}+2RS^-} \underset{}{\overset{\text{fast}}{\rightleftarrows}} \underbrace{\left[\mathrm{Fe^{2+}(RS^-)_2}\right]}_{\mathrm{I}} \qquad (1)$$

$$\left[\mathrm{Fe^{2+}(RS^-)_2}\right]+\mathrm{O_2} \longrightarrow \underbrace{\left[\mathrm{Fe^{3+}(RS^-)_2}\right]}_{\mathrm{II}}+\mathrm{O_2^{\cdot-}} \qquad (2)$$

and decaying by the chain propagating step:

$$\left[\mathrm{Fe^{3+}(RS^-)_2}\right]+\mathrm{RS^-} \longrightarrow \left[\mathrm{Fe^{2+}(RS^-)_2}\right]+\mathrm{RS\cdot} \qquad (3)$$

The kinetics of metronidazole reduction in the absence of oxygen are compatible within an analogous scheme but with the rapid formation of an intermediate coloured drug–iron–cysteine complex (III) following reaction (1).

$$\left[\mathrm{Fe^{2+}(RS^-)_2}\right]+\mathrm{RNO_2} \overset{\text{fast}}{\rightleftarrows} \underbrace{\left[\mathrm{Fe^{2+}(RS^-)_2{-}RNO_2}\right]}_{\mathrm{III}} \qquad (4)$$

Since for reaction (1) the equilibrium constant $K^e > 10^6$ the

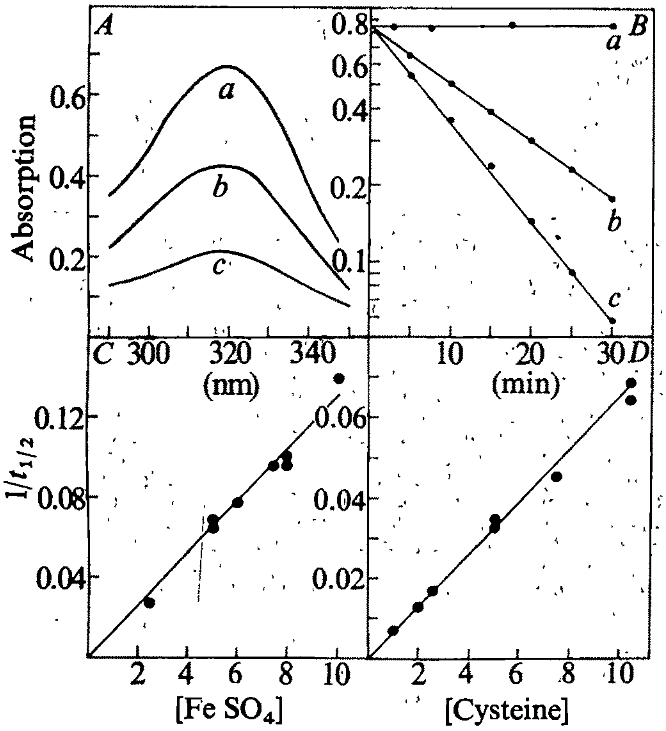

Fig. 1 *A*, Absorption spectra of ethyl acetate extracts taken at various times from nitrogen purged solutions originally containing cysteine (75 mM) ferrous sulphate (500 μM) and metronidazole (1 mM) pH $= 7.5$: *a* After 5 mins; *b*, 20 min; *c*, 40 min. *B*, Absorbance of extracts from solutions originally containing 0.1 M cysteine, metronidazole (1 mM) and ferrous sulphate. *a*, 0; *b*, 600 μM; *c*, 1 mM. *C*, Plot of reciprocal half lives (min^{-1}) for metronidazole removal against ferrous sulphate concentration ($\times10^{-4}$ M), 0.1 M cysteine. *D*, Plot of reciprocal half lives (min^{-1}) for metronidazole removal against cysteine concentration ($\times10^{-2}$ M), 500 μM ferrous sulphate.

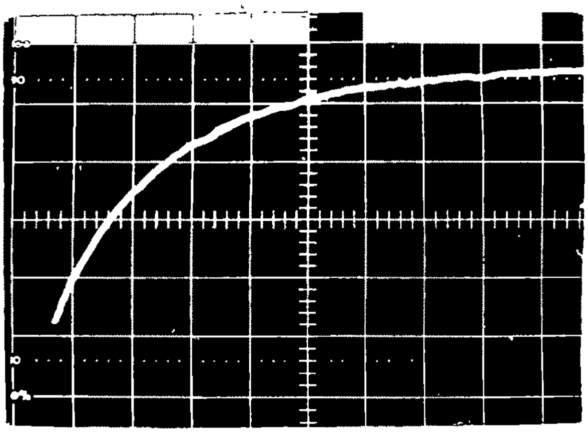

Fig. 2 Oscillogram obtained at 500 nm on mixing solutions containing metronidazole, cysteine and ferrous ion. (Final concentrations 25 mM, 0.1 M and 2 mM respectively, see text). Ordinate: transmission, 2.2% per large division; abscissa: time 1 s per large division.

rate-determining step for metronidazole reduction can be attributed to the reaction:

$$\left[\mathrm{Fe^{2+}(RS^-)_2{-}RNO_2}\right] + \mathrm{RS^-} \xrightarrow{\text{slow}} \left[\mathrm{Fe^{2+}(RS^-)_2}\right] + \mathrm{RNO_2^{\cdot -}} + \mathrm{RS\cdot} \qquad (5)$$

provided for reaction (4) the equilibrium constant is small, that is $K_c <$ or $\ll 0.1$.

Although the present studies provide no information concerning the fate of the radical products from reaction (5) it is probable that cysteine is formed according to:

$$\mathrm{RS\cdot} + \mathrm{RS\cdot} \longrightarrow \mathrm{RSSR} \qquad (6)$$

The subsequent reactions of $RNO_2^{\cdot -}$ however are less clear. It has been suggested that in the absence of oxygen the radical can react with nucleophilic centres and this could lead to cell death[16]. This would provide a unifying mechanism for its extremely selective cytocidal action on anaerobic organisms (aerobes ared amaged only at higher concentrations) and its radiosensitising effect on hypoxic but not on normally oxygenated tissue. Pulse radiolysis studies[16] have shown that the one electron redox potential of the oxygen–superoxide couple $E^\bullet$ ($O_2/O_2^{\cdot -}$) is greater than that of the metronidazole and metronidazole radical-anion couple $E^\bullet$ ($RNO_2/RNO_2^{\cdot -}$). Thus in the presence of oxygen the reaction:

$$\mathrm{RNO_2^{\cdot -}} + \mathrm{O_2} \rightarrow \mathrm{RNO_2} + \mathrm{O_2^{\cdot -}} \qquad (7)$$

can occur leading to removal of the damaging $RNO_2^{\cdot -}$ and the reformation of original drug. In the present studies this is in agreement with the lack of removal of metronidazole in oxygen purged solutions. In oxygen free solutions it is possible, also, that the reaction:

$$\mathrm{RNO_2^{\cdot -}} + \mathrm{RS^-} \rightarrow \mathrm{RNO_2^{\cdot -}} + \mathrm{RS\cdot} \qquad (8)$$

takes place leading to further cysteine destruction, although the possible inter-radical reactions:

$$\mathrm{RNO_2^{\cdot -}} + \mathrm{RS\cdot} \rightarrow \text{products} \qquad (9)$$

$$\mathrm{RNO_2^{\cdot -}} + \mathrm{RNO_2^{\cdot -}} \rightarrow \text{products} \qquad (10)$$

cannot be ruled out.

Whatever the fate of these radicals, however, it is clear that the reactions of metronidazole with cysteine and probably other sulphydryl compounds in the presence of ferrous ions are sufficiently fast to warrant consideration in the interpretation of the drug's mechanism of radiosensitising and chemotherapeutic action.

We thank the Cancer Research Campaign for financial support and May and Baker Ltd for metronidazole.

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Received March 10; accepted April 25, 1975.

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Survival of orally administered *E. coli* K12 in alimentary tract of man

VIEWS have recently been expressed that some aspects of genetic engineering research should not be allowed to continue because of their possible dangers to human health[1,2]. For example, objection has been taken to the laboratory practice of incorporating genetic regions of foreign DNA in bacterial plasmids and introducing them into organisms whose normal habitat is the alimentary tract, because such organisms may accidentally gain access to the alimentary tract of man, colonise it and/or transmit the plasmids during conjugation to the resident intestinal flora. Consequently, we have investigated this matter in regard to *Escherichia coli* K12, the intestinal organism most frequently used as the recipient of plasmids into which foreign DNA is incorporated, using a human volunteer in whose alimentary

Table 1 Concentration of different strains of *E. coli* K12 in faeces following oral administration

K12 strain	No. of viable organisms ($\log_{10}$ per g faeces voided on the following days after administration) 1	2	3	4	5
Experiment 1					
711*amp*ʳ	7.3	5.5	4.5	3.0	0
JRW *nal*ʳ	6.2	5.0	4.0	2.0	0
328Chl⁺	6.2	4.5	3.0	2.0	0
Proto *rif*ʳ	6.4	4.7	3.8	0	0
C600*str*ʳ	6.2	4.5	3.0	0	0
J5 Neo⁺	5.6	5.3	4.3	0	0
410*spc*ʳ	5.0	0	0	0	0
Resident *E. coli*	7.5	7.2	8.0	6.9	6.4
Experiment 2					
200*rif*ʳ	6.6	5.0	3.5	2.0	0
C600*nal*ʳ	6.5	5.1	0	0	0
Proto *amp*ʳ	6.0	4.0	4.2	2.2	0
410*spc*ʳ	6.0	3.3	0	0	0
711*str*ʳ	4.8	4.2	3.2	0	0
Resident *E. coli*	7.5	7.0	ND	ND	ND
Experiment 3					
711*nal*ʳTet⁺F⁺,I⁺ and A2⁺*	7.3	5.3	3.7	1.3	0
711*amp*ʳ	7.2	5.7	3.7	1.9	0
Proto *amp*ʳ	5.2	<4.0	<2.0	0	0
H123 *rif*ʳ†	8.2	7.8	6.5	3.4	2.4
Resident *E. coli*	5.7	6.5	6.9	6.4	6.4

Each strain was administered in doses of approximately $\log_{10}$ 9.0 viable organisms of an aerated broth culture that had been incubated at 37 °C for 24 h. 0 = $<\log_{10}$ 1.0. Viable *E. coli* counts were performed on the faecal specimens within 0.5 h of being voided, by the method of Miles and Misra[8], modified to permit the estimation of low concentrations of organisms. Ordinary MacConkey's agar was used for enumerating the total *E. coli*, and this medium containing antibiotics (20 µg ml^{-1}) for enumerating the different kinds of antibiotic-resistant *E. coli*. When the majority of the *E. coli* organisms in a specimen was not expected to be the resident strain, which was sulphonamide resistant, the latter was counted on Diagnostic Sensitivity Test Agar (Oxoid) containing 50 µg ml^{-1} chlorsulphapyridiazine In experiment 3, Tet transfer from the Tet⁺711 strains to the resident strain was sought by inoculating the faecal specimens heavily on to plates of MacConkey's agar containing tetracycline and applying sulphonamide disks to their surfaces; MacConkey's agar containing tetracycline and rifampicin was used for detecting transfer to strain H123.

*This was a mixture of three strains possessing three different transfer factors, F, I or A2.

†This was a strain of *E. coli*, not K12, recently isolated from the faeces of a healthy human being.

ND, Not determined.

Table 2 Concentration of *E. cyli* K12 711nal^r organisms in the faeces following oral administration at different dose levels

Approximate no. of viable organisms administered ($\log_{10}$)	No. of viable organisms ($\log_{10}$ per g faeces voided on the following days after administration) 1	2	3	4
9.0	7.7 6.5 5.3	6.5 4.5 5.0	0 1.0 0	0 0 0
	3.5 3.2 —	2.8 3.2 —	2.0 2.7 —	0 0 —
8.0	4.0 3.9 3.5	3.3 2.7 2.5	1.2 0 1.3	0 0 0
7.0	2.7 1.3 0	0 1.0 0	0 0 0	0 0 0
6.0	0 0 0	0 0 0	0 0 0	0 0 0
9.5*	7.3 5.2 5.2	5.5 4.5 4.5	3.7 2.8 1.3	1.3 0 0
	5.0 4.3 4.3	4.2 3.3 4.2	0 2.0 0	0 0 0

Each dose level was tested on three to six occasions; the results for each test at each dose level occupy the same relative position in each column, for example those found on days 1, 2, 3 and 4 for the first dose level of 9.0 tested were 7.7, 6.5, 0 and 0, respectively.

*Equal mixture of organisms of *E. coli* K12 711Tet$^+$ possessing transfer factors F, I and A2.

For other details see Table 1.

tract transfer of plasmids coding for antibiotic resistance (R factors) had previously been demonstrated following the consumption of wild strains of antibiotic-resistant *E. coli*[3]. Even when much higher concentrations of *E. coli* K12 organisms than would be expected[1] to be accidentally consumed in a laboratory were taken, they did not colonise the alimentary tract but organisms of some strains survived better than others; no evidence of transfer of plasmids from these K12 organisms to the resident *E. coli* of the alimentary tract was found.

Seven mutants of K12 requiring different nutrients for growth, and a prototrophic form of this strain, Proto, were studied. To determine the number of organisms of several of these strains in the same specimen of faeces, mutants resistant to different antibiotics were prepared from them. These included nalidixic acid (nal^r), streptomycin (str^r), spectinomycin (spc^r), ampicillin (amp^r) and rifampicin (rif^r). Two of the K12 strains, 328 and J5, were differentiated by their possession of plasmids determining resistance to chloramphenicol (Chl+) or to neomycin (Neo+). Some of the K12 strains were also differentiated by the inability of some of them to ferment lactose (lac^-). Three of the strains were also made thymine-dependent (thy^-) because thy^- salmonella organisms had been found to be poorer colonisers of the alimentary tract than thy^+ ones (H.W.S. and J. S. Tucker, unpublished). A plasmid determining tetracycline-resistance (Tet) was implanted in a nal^r mutant of one of the lac^- K12 strains, 711, using one or other of three transfer factors, F (refs 4 and 5), I (Idrd 16(I), (ref. 6) and A2 (ref. 7); the resulting strains, 711 nal^r Tet$^+$F$^+$, 711 nal^rTet$^+$I$^+$ and 711 nal^rTet$^+$A2$^+$ were used for studying the possibility of plasmids being transferred from ingested *E. coli* K12 organisms to the *E. coli* resident in the alimentary tract of the volunteer. In *in vitro* mating studies, using the method of Smith[8], all three strains could transmit Tet to the majority of *E. coli* K12 recipient organisms; in the Tet$^+$F$^+$ strain, Tet and F were linked. The resident *E. coli* during the whole study were dominated by one strain that possessed a transmissible plasmid determining sulphonamide resistance; it was usually present in concentrations of 10^6–10^8 viable organisms per g faeces.

The results of three experiments in which groups of antibiotic-resistant mutants of the eight different *E. coli* K12 strains were consumed simultaneously, each in doses of approximately $\log_{10}$ 9.0 viable organisms, and their concentrations in the faeces subsequently estimated are summarised in Table 1. None of the strains persisted in the faeces for longer than 4 d; the highest concentrations were found on the first day after consumption, progressively decreasing concentrations being found on subsequent days until the organisms were no longer isolated; some strains, notably 410 spc^r, persisted more poorly than others. By contrast, the high concentrations of organisms of the resident *E. coli* strain remained relatively constant throughout the three experiments. A human faecal *E. coli* strain, H123, was included in the third experiment. At each daily examination it was present in much higher concentrations than any of the K12 strains and it persisted in the faeces for 7 d. In this experiment there was no evidence of transfer of the tetracycline plasmid to strain H123 or to the resident *E. coli* strain from the three Tet$^+$711nal^r strains, 711Tet$^+$F$^+$, 711Tet$^+$I$^+$, and 711TET$^+$A2$^+$. In *in vitro* studies these three strains transferred their tetracycline resistance plasmids to H123 and the resident *E. coli* strain at approximately the same rate, about one in 500 organisms of these strains receiving the plasmid.

The results of consuming on three to six occasions at each of four different dose levels 711nal^r (one of the K12 strains that survived best in the alimentary tract, Table 1), are summarised in Table 2. In general, the larger the dose consumed the greater were the concentrations of organisms found in the faeces. Considerable variations in faecal concentrations were, however, sometimes found when the same number of organisms were consumed on different occasions. For example, the highest concentration of 711nal^r organisms found in the faeces following the consumption of $\log_{10}$ 9.0 organisms was $\log_{10}$ 7.7. When the same dose was consumed on another occasion the highest concentration found was $\log_{10}$ 3.2. Very few organisms

Table 3 Concentration of organisms of three thy^- and one thy^+ K12 strain in faeces following oral administration at different dose levels

Approximate no. of viable organisms administered ($\log_{10}$)	Strain	No. of viable organisms ($\log_{10}$ per g faeces, voided on the following days after administration) 1	2	3	4
10.0	410$spc^r thy^-$	4.8	0	0	0
	Proto $amp^r thy^-$	6.2	3.5	3.0	0
	711$str^r thy^-$	6.5	5.0	3.4	0
	711$nal^r thy^+$	7.8	6.4	4.4	2.7
	Resident *E. coli*	7.5	6.9	6.9	6.7
9.0	410$spc^r thy^-$	2.7 3.2 0	0 0 0	0 0 0	0 0 0
	Proto $amp^r thy^-$	2.7 2.8 2.2	1.3 2.4 0	0 0 0	0 0 0
	711$str^r thy^-$	4.3 4.0 3.2	3.2 2.8 2.3	0 0 0	0 0 0
	711$nal^r thy^+$	5.2 6.2 4.0	4.4 5.7 3.2	1.6 4.2 2.6	0 3.4 1.8
	Resident *E. coli*	7.0 7.2 6.7	6.2 7.0 5.7	6.4 7.2 6.7	6.7 6.5 6.3
8.0	410$spc^r thy^-$	1.6 0 0	0 0 0	0 0 0	0 ND 0
	Proto $amp^r thy^-$	3.5 2.0 2.0	1.9 0 0	0 0 0	0 ND 0
	711$str^r thy^-$	3.3 2.3 2.0	2.3 1.3 0	0 0 0	0 ND 0
	711$nal^r thy^+$	5.2 4.4 3.5	4.5 3.7 2.0	2.5 3.0 1.7	1.6 ND 0
	Resident *E. coli*	7.3 7.7 7.7	7.5 7.4 7.3	7.8 7.7 7.0	7.5 ND 6.5

For other details see Tables 1 and 2.

All culture media used in these experiments were supplemented by the addition of 50 µg ml^{-1} thymine.

were found in the faeces following the consumption of $\log_{10}$ 7.0 viable organisms of this strain and none was found when $\log_{10}$ 6.0 was consumed even though the cultural methods used enabled the isolation of organisms present in concentrations of 10 per g of faeces. In this experiment, mixtures containing $\log_{10}$ 9.0 viable organisms of each of the three 711*nal*ʳTet⁺ strains possessing transfer factors F,I or A2 were consumed on three separate occasions; transfer of the tetracycline plasmid to the resident *E. coli* strain was never observed.

The ability of three thymine-dependent (*thy*⁻) K12 strains and the *thy*⁺711*nal*ʳ strain to survive in the alimentary tract of the human volunteer are compared in Table 3. At all dose levels tested, the *thy*⁻ strains, especially strain 410 *spc*ʳ, survived more poorly than the *thy*⁺711*nal*ʳ strain.

The pattern of faecal excretion of the K12 organisms in all three experiments in which the highest concentrations of organisms were found on day 1 after ingestion, then progressively lower ones on subsequent days and none after day 4 suggested that in the main, the organisms were simply passing through the alimentary tract. The actual concentrations found in the faeces on some occasions indicated that the process was often accompanied by high mortality. The failure to demonstrate transfer of the tetracycline resistance plasmid from the 711*nal*ʳ organisms possessing the highly efficient transfer factors F,I and A2 to the resident *E. coli* flora was not unexpected because experiments in animals[9] had shown that to obtain substantial transfer of R factor required the use of organisms that were not only efficient donors and recipients but also good colonisers of the alimentary tract. Previous work using more robust strains of *E. coli* than K12 (refs 3, 10–12) has also demonstrated the difficulty of transferring R factors experimentally in the human alimentary tract.

The present work is open to the criticism that it was done on only one human being. Quite different results might have been obtained in other human beings. Even so, it is probable that the relative ability of different K12 strains to survive in the alimentary tract would be the same in most human beings. Note that when several strains were tested on the same occasion some, especially 410*spc*ʳ*thy*⁻, survived more poorly than others in the alimentary tract of the human volunteer. Everything else being equal, it would therefore seem prudent to use strains such as these in potentially dangerous genetic engineering experiments, unless ones that survive even more poorly can be found.

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Received March 10; accepted April 28, 1975.

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Viability of, and transfer of a plasmid from, *E. coli* K12 in the human intestine

THE controversy over the ability of *Escherichia coli* K12 to survive in the human gut and to transfer plasmids to the indigenous flora, arose in relation to proposed research on bacterial plasmids and other genetic agents hybridised with DNA from organisms unrelated to the normal hosts of the plasmids or to the plasmids themselves. As the bacterial strain used in most studies of microbial genetics is *E. coli* K12, experiments were designed to investigate its viability, and its capacity for plasmid transfer to the resident *E. coli*, in the human intestine. A preliminary summary of our observations was presented to the Working Party headed by Lord Ashby[1].

The K12 strain of *E. coli* was isolated from the faeces of a convalescent diphtheria patient in 1922, probably at Stanford Hospital, California. It was incorporated in the stock collection at Stanford University in 1925, and was given the catalogue designation K12. The question of its capacity for survival in the human intestine has become important since the call for an embargo on work with hybrid plasmids[2,3], because the organisms into which these plasmids are usually introduced is K12. If it can survive in the human intestine long enough to transfer a hybrid plasmid to the resident enterobacteria, DNA of non-bacterial origin (xeno-DNA) in such plasmids might be transferred to intestinal or other human cells. If the xeno-DNA were derived from a pathogenic microorganism such as a virus, which could cause human epidemic infections or malignancy (the properties of communicability and oncogenicity might be combined), work on such plasmids might present unacceptable biohazards in the absence of reliable safeguards.

We have carried out preliminary experiments to examine the efficiency of plasmid transfer to wild *E. coli* and salmonellae of human origin. *In vitro* transfer experiments were carried out using the donor strain *E. coli* K12 F⁻*lac*⁻, carrying the hybrid F′ plasmid F-*lac*-T (where T is tetracycline resistance). This is a recombinant of F-*lac* with the T-Δ factor, and was produced intracellularly[4]. The T-Δ R factor was isolated from type 29 of *Salmonella typhimurium*[5]. As far as can be determined it has no molecular similarity with F-*lac*, and the two plasmids belong to different compatibility groups, F_I in the case of F-*lac*, and I_1 in that of T-Δ. F-*lac*-T behaves as an ordinary F′ factor. It is

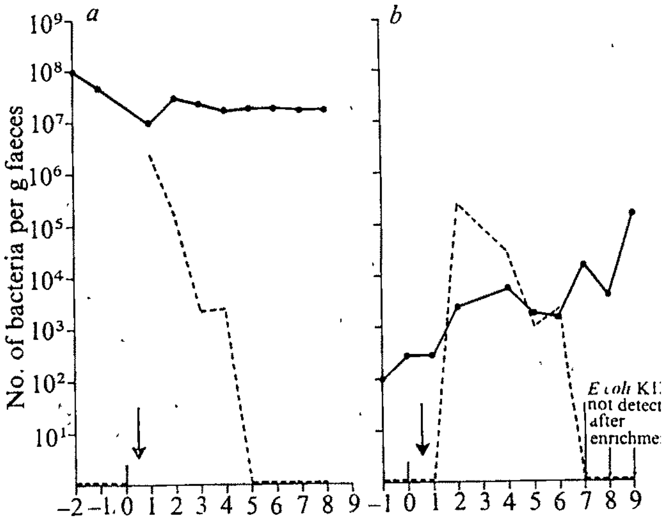

Fig. 1 Survival of *E.coli* K12 in human subjects after ingestion of: *a*, 3×10^{10} (subject 1); and *b*, 4×10^9 *E. coli* K12 F⁻*lac*⁻Nalʳ (subject 2). ●, Resident coliforms; dotted line, *E. coli* K12. Subjects were healthy adults aged 27 to 60 yr, three males and five females. Faeces were tested for 2 to 3 d before the experiment to exclude the presence of nalidixic acid-resistant resident *E coli.* None was found. Strain 14R519 in late exponential growth was administered (at arrow) in doses of about 10^3 to 10^{10} organisms in decimal steps. The dose was suspended in about half a pint of milk. Counts of 14R519 started 15 to 18 h later and continued until 3 to 4 d after the introduced strain was no longer detectable. Faecal specimens were emulsified 1 : 10 in saline Serial dilutions were plated on plain MacConkey agar for total faecal *E coli* counts. Counts were similarly carried out on MacConkey agar containing 40 µg ml⁻¹ nalidixic acid to detect 14R519. In six subjects enrichment of 14R519 was attempted by incubation of faeces in broth containing nalidixic acid as soon as the strain was no longer detected by direct plating. It was demonstrated by this method in one subject only. All isolates of 14R519 were tested for sensitivity to phage φ2 of Cuzin[6], which lyses K12F⁻ strains but very few strains of wild *E. coli*. All *lac*⁻Nalʳ isolates were sensitive to this phage. Strain 14R519 was not detected in the faeces of subject 8, who ingested 10^3 organisms.

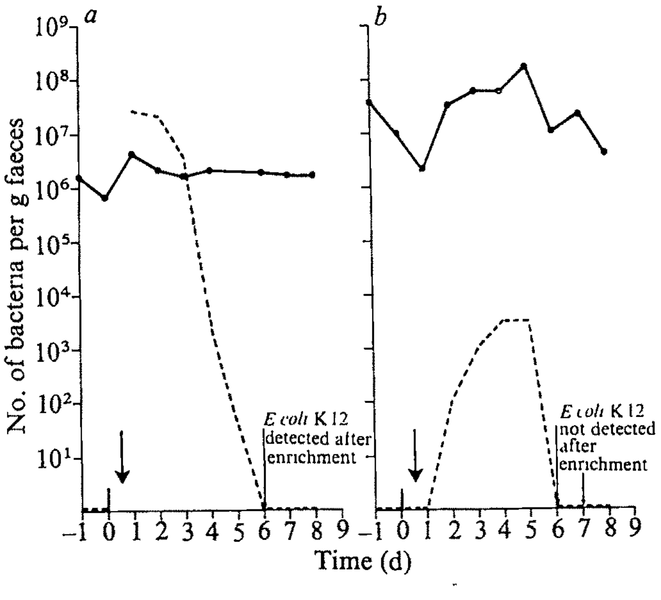

Fig. 2 Survival of *E. coli* K12 in human subjects after ingestion of: *a*, 3×10^8 (subject 3); and *b*, 9×10^6 *E. coli* K12 F$^-$*lac*$^-$ Nal^r (subject 4). Key and methods as in Fig. 1.

derepressed and has the same transfer kinetics and compatibilities as the F-*lac* factor. It has been assumed that only the T region of T-Δ is present in F-*lac*-T, but this awaits detailed confirmation.

Recipient strains used in the *in vitro* experiments were: *E. coli* EC36 (a wild drug-sensitive strain of human origin, and a fair recipient of the F factor); *E. coli* EC1 (a human drug-sensitive strain and a poor recipient of F); *S. agama* (a relatively rare serotype in man—about 0.3% of total annual human salmonellosis in Britain—and a fair recipient of F); *S. typhimurium* phage type 36 (*S.typhimurium* is the commonest human and animal salmonella in most countries, type 36 being a rather poor recipient of F). There are very few good recipients of the F factor: K12 is one of these, and it is also an excellent recipient of F-*lac*-T. A K12 line which is *lac*$^-$ and nalidixic acid-resistant (K12 *lac*$^-$Nal^r), numbered 14R519, was therefore used as a control recipient in these experiments.

Table 1 shows the lowest numbers of K12 *lac*$^-$ (F-*lac*-T) donor cells from which transfer of F-*lac*-T to each recipient strain was observed after 2, 6 and 20 h of mating. The donor dosage needed was less for the fair recipients EC36 and *S. agama* than for the poor recipients EC1 and *S. typhimurium* type 36, but transfer of F-*lac*-T was poor throughout, except in *S. agama*, where a dose of < 13 donor cells was adequate after 20 h. There was little spread of F-*lac*-T through the recipient population. In contrast to the results obtained with the wild *E. coli* and salmonellae, transfer of F-*lac*-T to 14R519 was unrestricted from the smallest donor dose, which was five organisms. Free spread of F-*lac*-T in the 14R519 population was also observed, unlike the wild *E. coli* and salmonellae, where spread was minimal. We conclude therefore that transfer of the hybrid F-*lac*-T plasmid from K12 to wild enterobacterial strains is probably poor in general.

Table 1 Quantitation of plasmid transfer to enterobacterial strains

Recipient strains	Minimum number of K12 *lac*$^-$ (F-*lac*-T) ml^{-1} to detect transfer to recipient in: 2 h	6 h	20 h
E. coli EC36	3×10^6	3×10^5	2×10^3
E. coli EC1	3×10^7	3×10^6	2×10^4
S. agama	7×10^6	2×10^5	<13
S. typhimurium type 36	$>10^8$	$>10^8$	10^3
E. coli K12 *lac*$^-$Nal^r 14R519 (control)	<5	<5	<5

Bacto-dehydrated nutrient broth (Difco) was used in a concentration of 2% for routine incubation of bacterial cultures and mating mixtures. Oxoid MacConkey agar No. 3 was used for detecting transfer to the recipient strains. Selection for F-*lac*-T transfer to recipients was exercised with tetracycline, of which 10 µg ml^{-1} was incorporated in the MacConkey agar. Counter-selection against the K12 donor was effected by incubating the mating mixture with high titre phage T6, and spreading undiluted colicin E$_2$ on the plates. When the recipient was 14R519 the donor K12 strain was eliminated by incorporating 40 µg ml^{-1} nalidixic acid in the medium. About 10^1 to 10^8 organisms of the donor strain were added to a culture of each recipient in late exponential growth in nutrient broth.

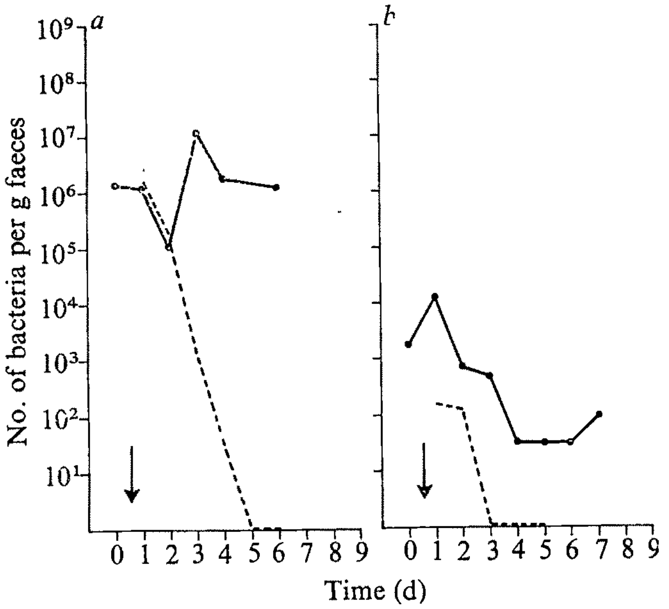

Fig. 3 Survival of *E. coli* K12 in human subjects after ingestion of: *a*, 3×10^6 (subject 5); and *b*, 3×10^5 *E. coli* K12 F$^-$*lac*$^-$Nal^r (subject 6). Key and methods as in Fig. 1.

To determine survival of K12 *in vivo*, *E. coli* K12 *lac*$^-$Nal^r strain 14R519 was used. Figures 1–4 show that *E. coli* K12 can survive for some days when introduced into the human gastrointestinal tract. Starting with the highest dosage the minimum survival periods were as follows: 10^{10}, 4 d; 10^9, 6 d; 10^8, 6 d; 10^7, 5 d; 10^6, 4 d; 10^5, 2 d; 10^4, 3 d. The shortest survival duration was 2 d (dose 10^5) and the longest, 6 d (doses 10^8 and 10^9). Although the larger doses gave in general the longest survival times, this was not directly dose related, as the results with subjects 1 to 5 show.

In subjects 3 and 5 at least, multiplication of 14R519 probably occurred. If it is assumed that the organism is uniformly distributed throughout the faeces when the count is stable for a minimum of 24 h, and that the contents of the adult large intestine are of the order of 1 l, the total count of 14R519 in the large bowel during at least 24 h exceeded the dose initially ingested. The distribution of 14R519 could be monitored only at 15 to 24 h intervals, so that the multiplication can only be postulated. Nevertheless it is a reasonable extrapolation from our results.

To transfer a hybrid F′ plasmid *in vivo*, the donor strain used was K12 *lac*$^-$ (F–T), Enteric Reference Laboratory No. 35R169, the F–T plasmid being derived from the F-*lac*-T hybrid[4] by loss or inactivation of the *lac* operon. The subject used for this experiment was a healthy adult male aged 29 yr, who had no tetracycline-resistant organisms in his faeces. Figure 5 shows that the resident *E. coli* count was 10^6 to 10^7 g^{-1} and the donor strain 35R169 persisted for 4 d. Transfer of F–T occurred at a very low level: about 20 *lac*$^+$F–T$^+$ organisms per g faeces were present 24 h after the donor strain was ingested. By the time the next specimen was tested 24 h later they had disappeared, and were not subsequently detected. Although we cannot absolutely exclude the possibility that *in vitro* transfer of F–T had occurred when the faeces were emulsified in saline for plating, the precautions taken to prevent this—addition of phage T6, colicin E_2 and tetracycline to the saline—make it unlikely. We believe that the appearance of F–T in the resident *E. coli* was the result of *in vivo* plasmid transfer. Moreover, if tetracycline had been administered during the experiment, the transfer frequency of F–T to the resident flora would probably have been dramatically increased[7,8]. We deliberately avoided this to preclude the possibility of biasing the result.

Unfortunately, it is only too clear that *in vivo* plasmid transfer is occurring constantly in man and animals. The prevalence of enterobacteria of all sorts, rich in R factors and other plasmids, bears witness to this, a state which is the result of the global pressure of antibiotics.

The results of our experiments are reasonably clear cut. Wild human *E. coli* and salmonellae are rather poor recipients of the F-*lac*-T hybrid. K12 can survive for a few days in the human intestine and may even multiply for a short while, but it is ultimately eliminated. The F–T hybrid can almost certainly be transferred to the resident *E. coli* in the human intestine. In the absence of antibiotic selection, however, *in vivo* transfer of F–T has a very low frequency, and F–T+ resident organisms do not persist, nor does the plasmid spread.

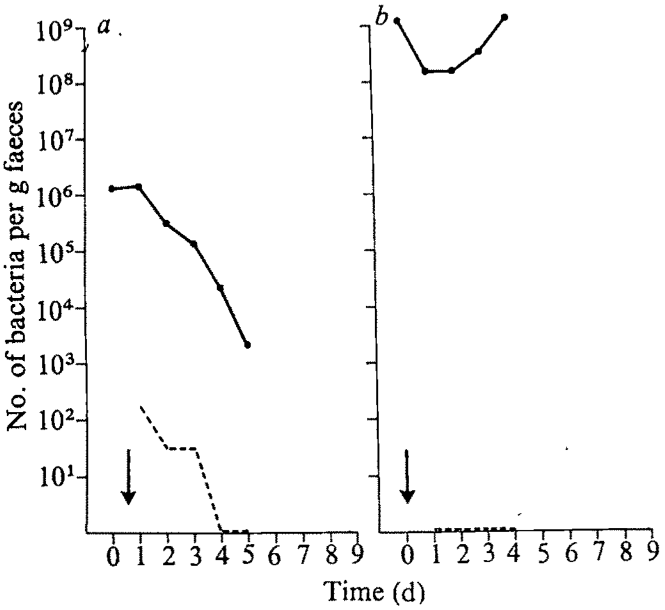

Fig. 4 Survival of *E. coli* K12 in human subjects after ingestion of: *a*, 3×10^4 (subject 7); and *b*, 3×10^3 *E. coli* K12 $F^-lac^-Nal^r$ (subject 8). Key and methods as in Fig. 1.

The results show therefore that K12 can survive in the human intestine and can transfer hybrid plasmids to the resident *E. coli*[1]. The fact that F-*lac*-T and F–T are purely bacterial hybrid plasmids probably does not prejudice the issue, since the transfer mechanism depends, not on the nature of the xeno-DNA in a plasmid, but on the transfer factor itself, the F factor in the present instance. The low F–T transfer frequency *in vivo* even when the dose of K12 was 10^{10} organisms, indicates the remoteness of the possibility of such an event occurring by accident, which would probably involve the ingestion of only a few hundred or thousand bacteria. Obviously, more experiments along these lines need to be done, but our present findings suggest that in most instances experiments involving K12-carrying hybrid plasmids can safely be carried out with the exercise of the simple but effective techniques used in laboratories handling organisms such as the typhoid bacillus and similar pathogens. It is in any event an advantage for all who work with bacteria to be trained in such techniques, which not only protect against accidental infection but also add reliability to the experimental findings.

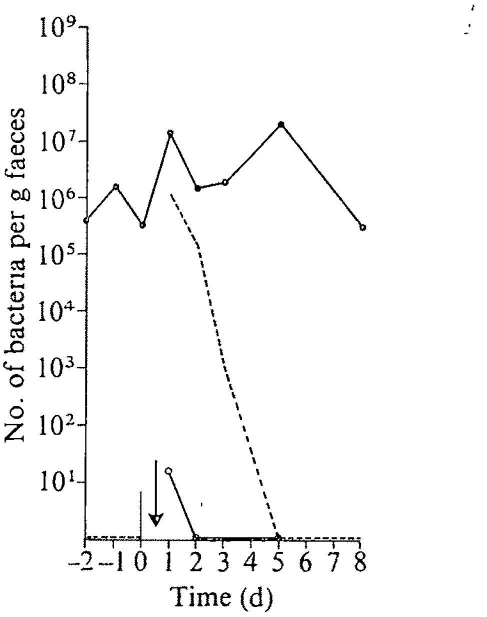

Fig. 5 Survival of K12 in a human subject (9) after ingestion of 10^{10} *E. coli* K12 lac^-(F–T). ●, Resident coliforms; dotted line, *E. coli* K12; ○, resident coliforms carrying F–T. Strain 35R169 was administered in a dose of 10^{10} organisms (at arrow) in half a pint of milk in early evening. The faeces were examined the following morning, and daily thereafter. The organisms monitored were: the resident *E. coli*; 35R169; and the resident *E. coli* carrying the F–T plasmid. Faeces were emulsified in saline to a dilution of 1 : 10. Unchanged resident *E. coli* were counted by serial dilution on ordinary MacConkey agar plates. Strain 35R169 was counted by a similar technique on MacConkey agar containing 10 µg ml^{-1} tetracycline. Resident *E. coli* carrying the F–T plasmid were detected on MacConkey agar containing tetracycline. To minimise the possibility of the result being affected by *in vitro* transfer to the resident *E. coli* while the faeces were being suspended in saline, phage T6, colicin E_2 and tetracycline were incorporated in the saline used for suspension. Colicin E_2 was also spread on the MacConkey agar before mating mixtures were plated further, to ensure against survival of the donor strain. No donor colonies survived this selection. About 1 ml of the faecal suspension was plated, in 0.2 ml quantities on five plates, for the detection of F–T transfer.

If there is reason to suspect that the danger is of a higher order, the safety measures must be scaled up accordingly. Moreover, additional safeguards can be imposed on both K12 (or other host strains) and hybrid plasmids by the induction of mutations that would be lethal except in the desired experimental conditions.

I thank members of my staff who volunteered to be 'guinea pigs' in these experiments. This paper was presented at the International Conference on Recombinant DNA Molecules, Asilomar, Pacific Grove, California (February 24–27, 1975).

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Received April 1, accepted May 1, 1975.

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Plasmid RP4 as a vector replicon in genetic engineering

THERE is now considerable interest in the construction of plasmids containing DNA fragments from a variety of bacterial genera, for purposes of studying the expression of the genetic potentialities of the incorporated bacterial DNA in different bacterial hosts. We report that the self-transmissible plasmid RP4 has only one site susceptible to cutting by the enzyme *Eco*RI, and should therefore be a very suitable bacterial DNA vector in such experiments.

The restriction endonuclease *Eco*RI, which cleaves double-stranded DNA to produce short, overlapping, self-complementary single-stranded ends[1,2], has been used to generate complementary single-stranded sequences between the plasmid vector and the DNA to be incorporated into the plasmid. The plasmid and bacterial DNA fragments are allowed to associate by hydrogen bonding, and the 3′-hydroxyl and 5′-phosphate ends are then covalently linked by the action of the enzyme DNA ligase[2]. Experiments described so far have been limited by the use of plasmids that are not self-transmissible as the DNA vector: the expression of 'foreign' DNA could be studied only in *Escherichia coli*, into which the 'recombinant' plasmid was introduced by transformation[3-5].

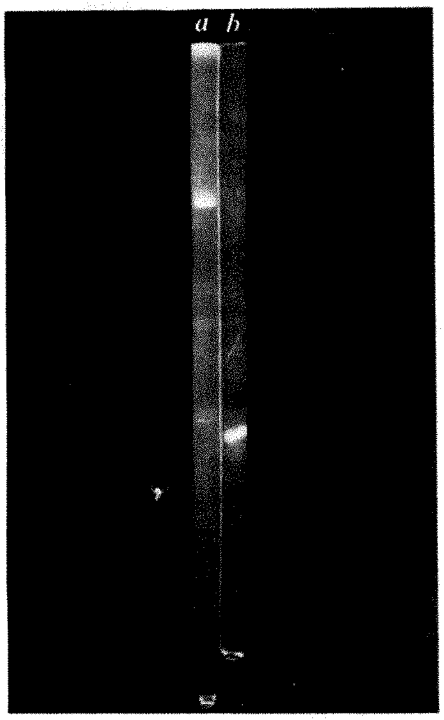

Fig. 1 Agarose gel electrophoresis of *Eco*RI-digested RP4 DNA. The covalently closed circular DNA tertiary form of plasmid RP4 was isolated using caesium chloride–ethidium bromide density gradient centrifugation[11,14]. Part (0.5 μg) of this DNA was digested with *Eco*RI enzyme and prepared for electrophoresis[5]. A second DNA sample was similarly prepared, except that *Eco*RI treatment was omitted. Electrophoresis was carried out vertically, in a downward direction, in 1% agarose gels held in glass tubes, 150 mm × 5 mm internal diameter, for 18 h at 2 mA per tube and 20 °C (ref. 13). Gels were removed from the holders and photographed under ultraviolet illumination through a red filter. Exposure was 4 min at F4.5 on Polaroid type 107 film. *a*, Covalently closed circular RP4 DNA; *b*, RP4 DNA after *Eco*RI endonuclease treatment.

The potential usefulness of the technique would be enhanced by the use of a plasmid that is transmissible, by conjugation, between many bacterial genera. Such manipulations fall into the purview of the Ashby Committee Report[6]; unless the 'foreign' DNA carries genes relevant to pathogenesis, no special hazard is to be anticipated and the techniques of containment usual in Public Health Laboratories would be appropriate.

Plasmids of incompatibility group P have a uniquely wide host range; they transfer to, and replicate stably in, many bacterial genera, including *E. coli*, *Proteus* spp., *Pseudomonas* spp., *Neisseria* spp., the photosynthetic *Rhodopseudomonas*, and the nitrogen-fixing *Azotobacter* and *Rhizobium* spp.[7-10]. Of the P group plasmids, RP4 has been most extensively studied[7,11]. It has been shown that RP4 can incorporate 'foreign' bacterial and plasmid DNA which can be genetically expressed; Dixon *et al.*[12] introduced the nitrogen fixation genes, *nif*, from *Klebsiella pneumoniae* into RP4 ,and an RP4 derivative has been isolated into which trimethoprim and streptomycin resistance genes have been transposed from a plasmid of the I incompatibility complex (unpublished). These plasmids were constructed by *in vivo* genetic manipulations.

We now show that RP4 has only one site that is susceptible to cutting by the *Eco*RI endonuclease to produce linear monomeric DNA and, as it is probable that only one gene function

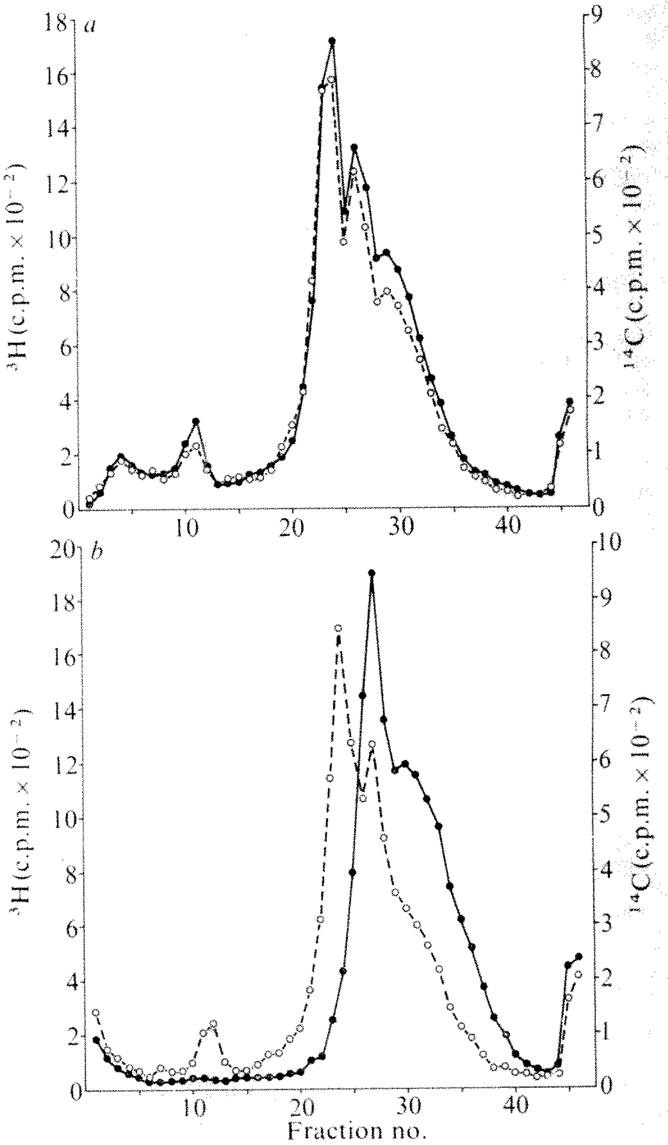

Fig. 2 Neutral sucrose gradient analysis of *Eco*RI-digested RP4 DNA. The covalently closed circular DNA tertiary form of plasmid RP4 was isolated as described in Fig. 1. This DNA spontaneously degraded to other tertiary forms on storage. For *Eco*RI digestion of DNA, 2 μl of endonuclease solution was added to 20 μl DNA solution (100 mM Tris-HCl, 50 mM NaCl, 5 mM $MgCl_2$, *p*H 7.5) and incubated at 37 °C for 30 min. Endonuclease activity was then destroyed by heating at 63 °C for 5 min[15]. DNA samples were analysed by sedimentation through a 4.6 ml 5 to 20% sucrose gradient[14] at 100,000*g* and 20 °C for 120 min. Sedimentation was from right to left. Fractions (0.1 ml) were collected directly on to glass fibre disks and, after drying, washing and redrying, were assayed for radioactivity: ●, ^{3}H; ○, ^{14}C. *a*, ^{14}C-labelled RP4 DNA plus ^{3}H-labelled RP4 DNA. *b*, ^{14}C-labelled RP4 DNA plus *Eco*RI-digested ^{3}H-labelled RP4 DNA.

has been destroyed, this molecule should make an ideal vector for genetic engineering.

RP4 has a molecular weight of 36 × 10^6 (P. T. Barth, personal communication). Initial experiments using agarose gel electrophoresis[5,13] showed that the covalently closed circular form of RP4 DNA was converted by digestion with *Eco*RI endonuclease to a single DNA band that migrated considerably faster than the untreated DNA (Fig. 1). We proved that *Eco*RI digestion converted RP4 DNA to the linear monomeric form, by comparing the rate of sedimentation of *Eco*RI-digested and untreated DNA through 5–20% neutral sucrose gradients. Figure 2*a* shows that untreated ^{14}C-labelled RP4 DNA and ^{3}H-labelled RP4 DNA cosediment; the bands that peak at fractions 11, 24 and 26 are the covalently closed, open circular and linear tertiary forms of RP4 DNA. The open circular and linear forms and the further degraded DNA (between fractions 28 and 38) were formed by spontaneous breakdown of the covalently closed circular DNA form on storage. After complete digestion by *Eco*RI, the ^{3}H-labelled RP4 DNA cosedimented only with the linear form of ^{14}C-labelled RP4 DNA (Fig. 2*b*). Using the equation $d_{oc}/d_{lin} = 1.0965 \times M^{0.01}$, where d_{oc}, d_{lin} are the distances sedimented from the meniscus by the open circular and linear tertiary forms of a plasmid of molecular weight M (P. T. Barth, personal communication); the molecular weight of the *Eco*RI-digested RP4 DNA was calculated to be 36 × 10^6.

We thank S. N. Cohen for the gift of *Eco*RI endonuclease; J. E. Davies for help with the electrophoresis technique; R. W. Hedges, P. T. Barth and Naomi Datta for discussions. The research was supported by grants to Naomi Datta from the MRC.

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Received April 10; accepted May 2, 1975.

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Carcinogenicity and mutagenicity tests of some hair colourants and constituents

CONSIDERATION of exposure to drugs or other chemicals is part of the normal haematological investigation of patients with neutropenia. As there have been occasional reports concerning hair colourants and haemopoietic disorders[1], we decided to investigate the long term effects of two 'semipermanent hair colourants' which came to our attention during routine questioning. A more extensive range of colourants (coded A–L) have been tested for mutagenic activity in bacteria. The effects of two constituent dyes have also been tested in bacteria and in human lymphocytes.

The colourants tested initially were J, one of a variety of proprietary preparations containing nitrophenylenediamines, and K, containing an azo-dye metal derivative and an aminonitrophenol. Both contain detergent and perfume. Their effects are being examined by repeated applications in aqueous acetone solution to the skin of A and DBA strain mice. Although topical applications were intended to provide a reasonable approximation to human usage, the mice groom themselves after treatment so that considerable amounts of colourant are probably ingested orally in addition to any that may be absorbed through the skin. No lesions have occurred on the treated skin.

In the DBA mice malignant tumours have, however, occurred so far in 5 out of 48 mice treated with compound J (26, 37, 41, 51, 61 weeks), two out of 32 mice treated with K (41, 47 weeks), and none of 32 controls. In the A strain mice malignant tumours have occurred in 4 out of 52 mice treated with J (48, 55, 57, 59 weeks), 3 out of 32 mice treated with K (38, 38, 72 weeks), and also in 3 out of 32 controls (61, 75, 77 weeks). All the tumours have been of the lymphoid system. In three mice treated with J, these were thymic lymphosarcomas but in the other animals the tumours were most commonly malignant lymphomas involving the spleen with infiltration of the liver and other organs. With most of the mice still on test, it is too early to evaluate the significance of these observations, especially in view of the well-known role of oncogenic viruses in the causation of malignant lymphomas in mice. It was, however, the recognition of these tumours at a relatively early age in the treated animals which stimulated further studies on the mutagenicity of these colourants.

Table 1 Mutagenicity assays of 11 hair colourants in *S. typhimurium* TA1538, with or without liver-microsomal activation

Test material	Induced *his*$^+$ revertant colonies per plate			
Benzo(α) pyrene (5 μg per plate)	−(S-9) 19 +(S-9) 188			
Hair colourant	Diluted (×10)		Diluted (×100)	
	−(S-9)	+(S-9)	−(S-9)	+(S-9)
A	7	0	7	0
B*	975	29	155	0
C*†	307	345	34	84
D*	807	614	157	57
E	18	0	13	0
F	0	0	8	0
G*	144	—	44	—
H	5	0	32	0
J*	1071	208	112	33
K*	15	388	28	228
L*	956	875	195	144

*Denotes levels of mutation considered significant in this assay system.

†Oxidising dye containing toluylenediamine.

Each hair colourant was diluted tenfold (v/v) in deionised water, filter-sterilised and a further tenfold dilution in sterile water was prepared. Samples (0.1 ml) of each dilution were assayed for their ability to induce reversion to *his*$^+$ in *S. typhimurium* TA1538 in the presence or absence of benzo(α)pyrene-induced rat-liver supernatant (S-9 mix) by the methods described by Ames *et al.*[2]. Benzo(α)-pyrene was used as a positive control for the metabolic activity of the S-9 mix. Plates were scored for revertant colonies after 3 d incubation at 37 °C. Values shown are induced *his*$^+$ revertants per plate (mean of two plates) after subtraction of spontaneous revertants (12 per plate without S-9, 45 per plate with S-9).

Carcinogenicity tests, particularly with weak carcinogens, are prolonged and expensive. There is great interest in short term tests, which depend on the reasonable correlations observed between carcinogenicity and mutagenicity[2], for screening substances present in the environment. We have tested colourants J and K, and nine similar products, for mutagenicity in bacteria. The organisms used were *Escherichia coli* WP2, WP2 *uvrA*, and WP2 *exrA*, all of which are *trp*$^-$ and revert to *trp*$^+$ by base substitution[3]; *Salmonella typhimurium* TA1535, TA1537, and TA1538, all of which are *his*$^-$, *uvrB*, and have defective lipopolysaccharide coats which render them highly permeable to many mutagenic carcinogens. TA1535 is a base-substitution mutant, and TA1537 and TA1538 are both frameshift mutants[4].

Table 2 Mutagenicity of 2-nitro-*p*-phenylenediamine and 4-nitro-*o*-phenylenediamine in *S. typhimurium* TA1538 with or without liver-microsomal activation

Dose applied	Induced *his*$^+$ revertants per plate 5 μg per plate −(S-9)	 +(S-9)	 50 μg per plate −(S-9)	 +(S-9)
Test compound				
2-NPPD	43	27	335	213
4-NOPD	133	188	883	727

A pour-plate technique was used for all mutagenicity assays. For the *E.coli* series, the top agar contained 5% nutrient broth, 5 μg ml^{-1} tryptophan, 0.6% NaCl and 0.6% agar. The *Salmonella* series was assayed as described in Table 1. Vogel–Bonner MEM[5], with 1.5% agar and 2% glucose, was used as bottom agar in all assays.

Negative results were obtained in those bacteria which revert by base-pair substitution (*E.coli* WP2 series, and *S. typhimurium* TA1535). Seven of the eleven colourants were strongly mutagenic in *S. typhimurium* TA1538 (Table 1), and of these, two (D and J) were weakly mutagenic in TA1537. Six colourants (B, C, D, G, J and L) did not require microsomal activation for mutagenic activity, and B and J were markedly less mutagenic in the presence of the S-9 liver fraction. K, however, was mutagenic only when activated by the S-9 fraction.

The two dyes, 2-nitro-*p*-phenylenediamine (2-NPPD) and 4-nitro-*o*-phenylenediamine (4-NOPD); (purity >97%), present in colourant J (both from Aldrich, Wembley, UK) were tested without purification, and found to be mutagenic in TA1538 (Table 2) and weakly mutagenic in TA1537. 4-NOPD was about three times more potent than 2-NPPD at equivalent doses. Microsomal activation was not required for this activity. Ames[6] has also found 4-NOPD to be mutagenic in *Salmonella*.

To determine whether the mutagenic colourants contained either or both of these dyes, each colourant was fractionated using thin-layer chromatography on silica gel (Polygram Sil N-HR/UV$_{254}$; Camlab, Cambridge) in diethylether–petroleum ether (4:1) using 2-NPPD and 4-NOPD as markers. This solvent resolved three to ten coloured spots from colourants B, C, D, G, J and L. A strip (1 cm wide) was cut from each chromatogram and the spots, and the colourless areas between, were cut out and assayed for mutagenicity by plating with TA1538. This technique showed that each of these colourants contained fractions of high mutagenicity corresponding in R_f and colour to 2-NPPD and/or 4-NOPD. In some cases, mutagenic activity was also associated with colourless areas near the origin. The identity of the mutagenic agent(s) in colourant K, which requires metabolic activation, has not been determined.

Table 3 Effect of 2-nitro-*p*-phenylenediamine on cultured human lymphocytes

	Total cells examined	Number of cells with aberrations B cells	 C cells	 Total abnormal
2-NPPD present from time zero (μg ml^{-1})				
75	100	10	1	11
50	100	8	1	9
25	100	5	0	5
2-NPPD present for 24 h before harvesting (μg ml^{-1});				
75	100	21	2	23
50	100	17	0	17
25	100	2	1	3
Control	100	3	2	5

Human peripheral blood lymphocyte cultures were collected at 48 h. Cells were stained with orcein and scored for aberrations but not fully analysed. The classification of Buckton and Pike[8] was used. B cells have chromosome or chromatid gaps or breaks only, whereas C cells have stable or unstable chromosome rearrangements

Many carcinogens and mutagens are known to be chromosome-breaking agents. When the colourants J and K were added to cultures of human peripheral blood lymphocytes[7], serious toxicity, presumably caused by detergent, was noted. No chromosome damage was observed when dilutions high enough to avoid this toxicity were used. A series of experiments using 4-NOPD at concentrations up to 100 μg ml^{-1} failed to show any chromosome damage in cultures incubated for 48 and 72 h when the dye was added at the start of incubation, or at 24 h or 4 h before collecting. Similar experiments with 2-NPPD at concentrations between 50 μg ml^{-1} and 100 μg ml^{-1}, however, showed a considerable number of chromosome and chromatid gaps and breaks (Table 3). At 100 μg ml^{-1}, mitotic delay and toxicity were evident, but it was still possible to show that up to 45% of the cells contained damaged chromosomes. Cultures treated with lower concentrations of 2-NPPD did not differ significantly from controls.

Although a significant degree of carcinogenicity in a cosmetic preparation would undoubtedly be regarded as quite unacceptable, the human relevance of mutagenic activity is much harder to assess, especially when there are additional uncertainties about amounts of constituents absorbed into the body. The short term toxicity of *p*-phenylenediamine in man is well established[1]. The nitrophenylenediamines are, however, less well studied and those we purchased carried a warning that their toxicological properties have not been fully investigated and that their handling or use may be hazardous. They are, nevertheless, widely used in hair colourants, some of which we find to be mutagenic and possibly carcinogenic. Our observations suggest a possible long term hazard and indicate the need for comprehensive toxicological evaluation of hair colourant constituents.

We thank the Cancer Research Campaign for financial support; Dr E. L. Jones for examining the histology of the mouse tumours and Ms V. Nash, C. Macpherson, K. Vause and C. Bushell for assistance.

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Received February 25; accepted May 5, 1975.

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Helper function of T7 protein kinase in virus propagation

A PROTEIN kinase, the genetic information for which is phage-coded, is induced after infection of *Escherichia coli* with either phage T7 or T3 (ref. 1). The protein kinase is important in the regulation of gene expression in that it inhibits the initiation of transcription by *E. coli* RNA polymerase and in that it restricts host-dependent transcription of the T7 genome to the early region[2]. T7 protein kinase phosphorylates the β′ subunit of *E. coli* RNA polymerase (this action provides an explanation for the effects on transcription) and other *E. coli* proteins[1,2].

From the above considerations, one would have expected that the absence of protein kinase would have serious effects on phage development. The contrary was found, however, and the burst size or latent period in infection did not depend on the presence of protein kinase[1,2]. Furthermore, one widely distributed T7 strain and its many derivatives was found to lack the gene for protein kinase[2]. Yet no growth disadvantages of these phages to protein kinase-synthesising T7 strains was observed during many years of laboratory cultivation. If the protein kinase gene was not needed, what made it survive during evolution?

The results presented here demonstrate that protein kinase is in fact required for phage development in suboptimal growth conditions. In those conditions, the absence of protein kinase can lead to a very low or nonexistent burst size. The two growth conditions which we found to date and which display an effect of a protein kinase deficiency are propagation in poor media, and propagation at elevated temperature.

The effects of poor media were demonstrated for the development of T7 in various *K12* strains. Protein kinase-deficient mutants of phage T7 (T7 K^-) gave only few, if any, progeny phages, when used to infect *E. coli K12* grown in minimal medium, whereas the wild type phage (T7 K^+) was propagated (Table 1). In rich medium, such as tryptone broth or tryptone yeast broth, T7 K^- gave as many infective centres as did T7 K^+ (Table 1). The property to select against protein kinase-deficient T7 in poor media was a general feature of all *K12* strains tested (Table 1), whereas other *E. coli* strains, such as *E. coli B* and *E. coli C*, propagated T7 K^+ or K^- with approximately equal efficiency regardless of whether they were grown in tryptone broth or minimal medium (Table 1). Since *E. coli B* is the most commonly used strain for propagating T7, the effects of kinase would not have been detected.

Table 1 Requirement of T7 protein kinase for propagation in minimal medium

Host	Infecting phage T7 K^+	Infecting phage T7 K^-
M9 medium		
E. coli K12		
514	9	0.6
W1655	1.5	0.15
C600	0.6	0.03
ED3736	13	3
YC3272	2	0.25
803	0.6	0.06
E. coli B		
B_{s-1}	16	14
BB	17	17
O11	1	2
E. coli C		
HF4704	1	0.6
HF4714	0.4	0.2
Tryptone broth		
B_{s-1}	112	80
514	25	29

Various *E. coli K12* strains and *E. coli B* strains were grown either in M9 medium (Fig. 1) or in tryptone broth (1% tryptone, 0.5% NaCl) and infected with T7 K^+ or T7 K^-, respectively (multiplicity of infection 10). Survivors were plated 3 min after infection. Infected cells were determined by the difference between input celis and survivors. At 5 min, non-adsorbed T7 were inactivated by antiserum. At 10 min, cells were diluted 1:1,000 and the number of progeny virus determined at various times thereafter. Burst size per infected cell was calculated and listed.

Strains used have the following properties: *W1655* (*E. coli K12*): $F_{\Lambda 33\text{-}43}$, met^-, RC^-; *C600* (*E. coli K12*)$_8$ B_1^-, thr^-, leu^-, su^+ ,$\Phi 80^R$; *ED3736* (*E. coli K12*): his^-, trp^-, lac^-, $T6^R$, str^R, gel^-, su^-; *IC3272* (*E. coli K12*); F^-, $lac_\Lambda \times_{74}$, gal^-, mal^-, λcrp^+, λ^R, $T6^R$, $T1^R$, B_1^-, his^-, trp^-, lys^-, str^R, su^-; *514* (*E. coli K12*): F^-, lac_Λ, trp^-, str^R; *803* (*E. coli K12*): gal^-, met^-, lac^-, r_K^-, m_K^-; $B_{s\text{-}1}$ (*E. coli B*):$uvrA^-$, su^-; *BB*: su^+ *derivative of E. coli B*; *011*: *su*: derivative of *E. coli B*; *HF4704* (*E. coli C*): $\Phi X174^s$, su^-, trp^-; *HF4714* (*E. coli C*): $\Phi X174^s$, su^+, trp^-.

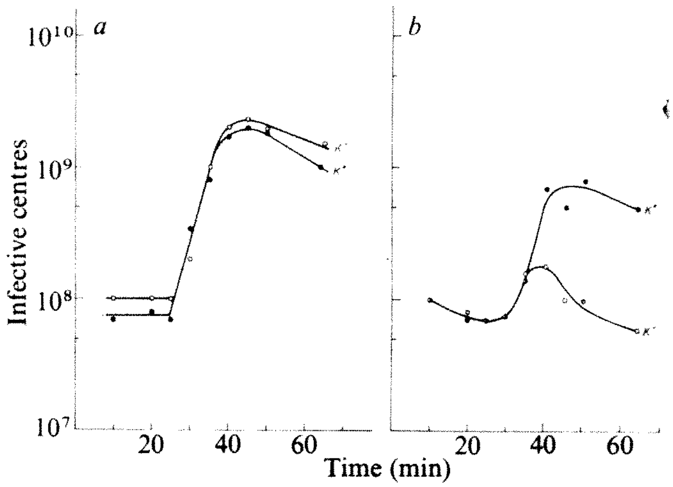


Fig. 1 Development of T7 with and without protein kinase in *E. coli K12* grown in minimal medium. *a*, *E. coli K12 514* and *b*, *E. coli* $B_{s\text{-}1}$ were grown at 30 °C in M9 medium ($Na_2HPO_4 \cdot 2H_2O$ (7.2), KH_2PO_4 (3), NaCl (0.5), NH_4Cl (1.0); and 0.4% glucose). At an optical density (600 nm) of 0.2, the cells were infected with either T7 K^+ (●) or T7 K^- (○) (isogenic pair as described in ref. 2) at a multiplicity of infection of 10. To remove non-adsorbed virus, antibodies were added at 5 min after infection. At 10 min after infection, part of the infected cells were diluted 1:10^3, 1:10^4, 1:10^5 and 1:10^6 and incubation at 30 °C continued. Aliquots (0.1 ml) of the dilutions were plated with indicator bacteria at various times. The undiluted part was used for the pulse-label experiment shown in Fig. 2. Surviving cells were plated at 3 min after infection.

The latent periods were identical with T7 K^+ and with T7 K^- when grown on *E. coli K12* minimal medium (Fig. 1). The reduction in T7 K^- progeny reflects the reduction in burst size per phage-developing cell (Fig. 1). In some strains this was less than one—the initial number of infective centres even decreasing with time, indicating that some fraction of cells did not develop any virus at all unless they were saved early on by plating on rich medium (data not shown).

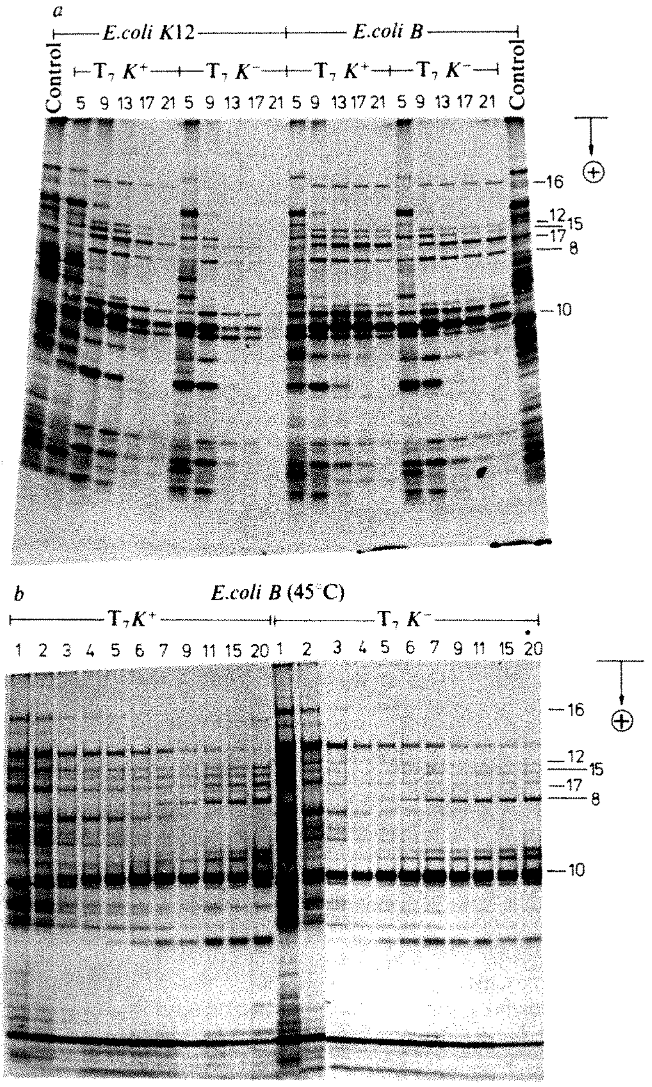
Associated with the inefficient development of protein kinase-deficient T7 in *E. coli K12* is a reduction in late viral protein synthesis at times beyond 14 min after infection. In pulse experiments with ^{14}C-labelled amino acids and subsequent analysis of the proteins in slab-gel electrophoresis the products of the T7 late genes 16, 12, 15, 17, 8, and 10 were hardly detectable during 16–20 min, whereas the control experiment with wild type T7 showed a maximal rate of synthesis of these proteins at this time (Fig. 2). In *E. coli* B_{s-1} (with the best burst size) late protein synthesis continues even further (Fig. 2). Thus, burst size seems to be directly related to the efficiency of late protein synthesis.

T7 K^- bacteriophage particles were less stable than T7 K^+ progeny after growth in *E. coli K12* in minimal medium. As indicated in the one-step growth curve (Fig. 1), 60% of T7 K^- were inactivated at 30 °C within 20 min. The instability of T7 K^- progeny cannot, however, account for all the difference between T7 K^+ and K^-; in an experiment in which newly-formed phages were allowed to adsorb to cells, the difference between the number of infective centres of T7 K^+ and T7 K^- was still apparent and similar to that shown in Fig. 1.

T7 protein kinase seems to promote late phage protein synthesis in *E. coli K12* grown in suboptimal conditions. A similar but weaker effect has also been observed in *E. coli B* (barely visible in Fig. 2*a*, but see evaluation in Fig. 6, ref. 2) but did not result in a measurable decrease in burst size. For both *K12* and *B*, large proteins were more sensitive to the lack of support from protein kinase than small proteins.

Growth of any *E. coli* host at elevated temperature also reduced the yield of protein kinase-deficient T7 and at 45 °C the burst size dropped to virtually zero (Fig. 3). This inhibition occurred in rich and poor media. The burst of T7 wild type was only slightly decreased. The difference in detectable phage

Fig. 2 The efficiency of late T7 protein synthesis during virus propagation in suboptimal conditions. Aliquots (0.2 ml) of infected cells from the experiments in Figs 1 and 3 (from the non-diluted culture) were removed at various times and pulse-labelled with ^{14}C amino acids (5 μCi ^{14}C protein hydrolysate) according to ref. 2. The samples from the experiment shown in Fig. 1 were pulse-labelled at 30 °C for 4 min (*a*), those of Fig. 3 at 45 °C for 1 min (*b*). After the pulse, incubation was continued with an excess of cold amino acids for 1 min; the cells were then collected, dissolved in sodium dodecyl sulphate buffer and the proteins resolved by polyacrylamide slab-gel electrophoresis (10в20% linear gradient). Autoradiograms of the dried gels are shown. The numbers on top of each position represent the beginning of pulse. Some T7 protein bands are marked by the numbers of the corresponding genes.



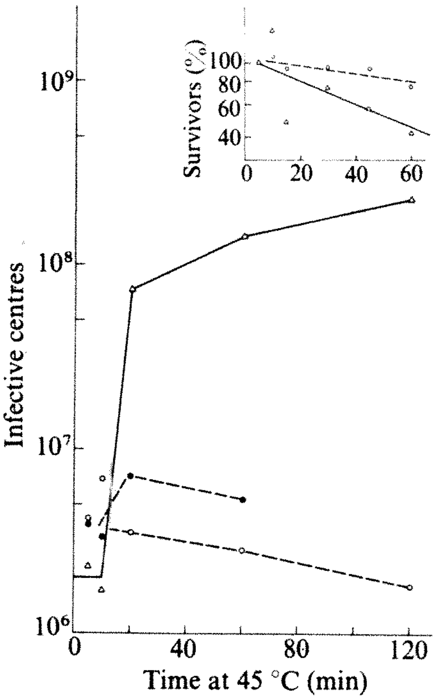


Fig. 3 Development of T7 K^- in *E. coli B* at elevated temperature. *E. coli* B_{s-1} were grown in M9 medium at 37 °C. At an optical density of 0.2 (600 nm) part of the culture was shifted to 45 °C, the other 44 °C. After an additional 5 min, 0.5 ml aliquots were infected with T7 K^+ (△, 45 °C; 44 °C not shown) or T7 K^- (○, 45 °C; ●, 44 °C) respectively, all at an approximate multiplicity of infection of 0.1. At 3 min, cells were diluted 1:10^4 into medium of the appropriate temperature and aliquots plated with indicator bacteria at various times (the plates were kept at 30 °C). The insert shows the survival of of T7 K^- (△) and T7 K^- (○) at 45 °C.

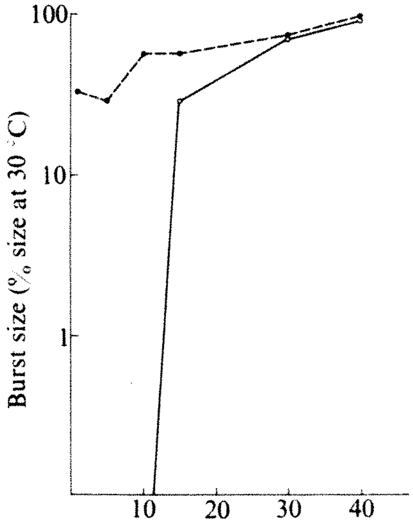


Fig. 4 Requirement for T7 protein kinase at 45 °C. Kinetics of occurrence of the heat-sensitive step. A culture of *E. coli* B_{s-1} in tryptone broth at 30 °C was infected with T7 K^+ or T7 K^-, respectively, at an absorbance of 0.2 (600 nm) and a multiplicity of infection of 0.1. At various times after infection, aliquots were diluted 1-10^4 into tryptone broth kept at 45 °C. Subsequent phage development was monitored and the burst size per infected cell determined. The burst size (% size at 30 °C) was plotted. ●, T7 K^+; ○, T7 K^- (data for 1-10 min were below 0.1%).

cannot be attributed to a difference in heat stability, as protein kinase-deficient phages were more heat stable at 45 °C than the wild type (Fig. 3), (the kinase mutation is the result of a small deletion and deletions have been found to be more heat stable[3–5]).

The effect of heat on viral growth occurs late in virus development. A shift from 30 to 45 °C reduced the virus output even when applied as late as 15 min after infection (Fig. 4). At that time, most DNA synthesis and late protein is already completed. In agreement with this observation the kinase mutation did not have any effects on the rate of synthesis of early and late proteins at 45 °C (Fig. 2). Although late T7 protein synthesis induced by T7 K^- was normal, no intracellular premature phage (not shown) and no release of mature phage was observed in these conditions (Fig. 3).

Our data suggest that the protein kinase was necessary for different steps in phage production at 45 °C and for growth in minimal medium with *K12* strains. At 45 °C, protein kinase stabilised an essential process late in viral development cycle, whereas in *K12* grown in M9 medium, protein kinase was needed for sufficient accumulation of late proteins. Experiments with T3 phage carrying a protein kinase mutation and with T3 wild type yielded similar results.

Our experiments indicate that the T7 and T3-specific protein kinase is required for the development of phage in suboptimal conditions. Outside the laboratory, where growth conditions are likely to be suboptimal, the protein kinase gene may have some survival value. Indeed, T7 and T3 phage collected from various fields all induced protein kinase (R. Goslich, unpublished). Thus, the natural environment has conserved the gene for protein kinase during evolution.

We thank W. Schreiber for assistance.

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Received February 27; accepted April 15, 1975.

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Seryl-tRNA synthetase and activation of the carcinogen 4-nitroquinoline 1-oxide

THE carcinogenic and mutagenic compound, 4-nitroquinoline 1-oxide (4NQO) and its reduced metabolite, 4-hydroxyamino-quinoline 1-oxide (4HAQO) bind covalently to cellular macromolecules such as nucleic acid and protein[1–7]. As 4HAQO rarely binds to nucleic acid in buffer solutions, it was suggested that a metabolic activation of 4HAQO is involved in the *in vivo* reaction. The presence of an activating enzyme has been demonstrated in extracts of rat ascites hepatoma cells and bakers' yeast[8,9]. The enzyme activates 4HAQO to bind to nucleic acid or protein in the presence of ATP, L-serine and Mg^{2+} (ref. 9). The nucleic acid adducts formed in the enzyme system show essentially the same chromatographic patterns as those of the purine adducts formed *in vivo*[1,6,8]. Moreover, the DNA lesions caused by 4HAQO binding in the *in vitro* enzyme system, like those lesions formed *in vivo*, can be repaired by the excision repair mechanism in *Bacillus subtilis*[10]. We have now purified 4HAQO-activating enzyme from bakers' yeast and report here that this enzyme is seryl-tRNA synthetase.

The enzyme was purified from commercial bakers' yeast to near homogeneity (Table 1, detailed procedures will be reported elsewhere). As reported previously, 4HAQO-activating enzyme requires ATP and L-serine for its binding activity[9] as well as Mg^{2+} and an sulphydryl protective reagent for maximal activity[8]. To characterise the reaction, we have analysed the *in vitro* reaction products by paper electrophoresis. As shown in Fig. 1, ^{3}H-AMP and ^{32}P-pyrophosphate were detected among the reaction products when ^{3}H-ATP and γ-^{32}P-ATP, respectively, were initially present. No significant conversion of L-serine was observed however. The mode of ATP degradation and the requirement for L-serine suggest that the reaction may be catalysed by seryl-tRNA synthetase similar to the well known amino acid activation catalysed by aminoacyl-tRNA synthetase in which degradation of ATP to AMP and pyrophosphate takes place if an appropriate amino acid is present.

After testing the purified enzyme for seryl-tRNA synthetase activity, we found that this activity was associated with 4HAQO-activating enzyme throughout the purification steps (Table 1). These two activities could not be separated by chromatography on DEAE–cellulose or Sephadex G-200 (Fig. 2) or by centrifugation on a sucrose density gradient. Both activities showed the

Table 1 Relative rate of 4HAQO-binding and seryl-tRNA synthesising activities during purification of 4HAQO-activating enzyme

Purification step	4HAQO binding*	Seryl-tRNA synthesis*
Antolysate	0.5	0.6
Acetone powder	1.0	1.0
Phosphocellulose	1.5	1.6
First ethanol precipitation	6.1	4.6
Second ethanol precipitation	34.6	28.8
DEAE–cellulose	97.8	105.4
DEAE–Sephadex	206	206
Hydroxylapatite	487	457

*Relative rate of specific activities.

Acetone-dried powder prepared from the autolysate of yeast[9] was extracted with 0.02 M potassium phosphate, *p*H 6.5, containing 1 mM EDTA and 1 mM 2-mercaptoethanol (buffer A). The extract clarified by centrifugation was passed through a phosphocellulose column, and enzyme in the unadsorbed fractions was precipitated by the addition of $Mg(OAc)_2$ (10 mM) and ethanol (0.3 volumes) at −20 °C. Enzyme was dissolved in buffer A containing 10 mM $Mg(OAc)_2$, treated with 30% ethanol at 37 °C for 10 min, and the precipitate was extracted with 0.02 M potassium phosphate, *p*H 6.5, containing 1 mM EDTA, 1 mM $Mg(OAc)_2$, 1 mM 2-mercaptoethanol and 5% glycerol (buffer B). The enzyme solution was loaded on a DEAE–cellulose column, eluted with 0.25 M potassium phosphate, *p*H 6.5, and dialysed against buffer B. The enzyme preparation was then chromatographed on a DEAE–Sephadex A-50 column eluting with a potassium phosphate linear gradient from 0.05 to 0.35 M, *p*H 6.5, containing 1 mM EDTA, 1 mM $Mg(OAc)_2$, 1 mM 2-mercaptoethanol and 5% glycerol. After dialysis against 0.02 M potassium phosphate, *p*H 7.0, containing 1 mM EDTA, 1 mM $Mg(OAc)_2$, 1 mM 2-mercaptoethanol and 10% glycerol (buffer C), enzyme was applied on a hydroxylapatite column and eluted with a potassium phosphate linear gradient from 0.02 to 0.3 M, *p*H 7.0, in the presence of 10% glycerol and 1 mM each of EDTA, $Mg(OAc)_2$ and 2-mercaptoethanol. Fractions containing enzyme were collected, dialysed against buffer C and stored at −20 °C. Activities in the final step were 0.43 μmol 4HAQO bound per mg protein per min and 0.53 μmol seryl-tRNA formed per mg protein per min. The reaction mixture (0.1 ml) for the 4HAQO-binding assay contained 1 mM ^{3}H-4HAQO (1.5 Ci mol^{-1}), 3 mM ATP, 10 mM L-serine, 3 mM $Mg(OAc)_2$, 1 mM DTT, 100 μg ml^{-1} bovine serum albumin, 20 mg ml^{-1} yeast RNA, 50 mM Bicine–KOH, *p*H 9.0, and enzyme. After incubation at 30 °C for 10 min, the reaction was stopped by the addition of sodium dodecyl sulphate solution followed by phenol extraction[8]. RNA was precipitated by the addition of ethanol, collected on Whatman GF/C paper disks and washed with cold TCA. Radioactivity remaining was measured in a liquid scintillation counter. The assay of seryl-tRNA synthetase activity was carried out in 0.05 ml containing 0.1 mM U-^{14}C-L-serine (2.0 Ci mol^{-1}), 3 mM ATP, 10 A_{260} units ml^{-1} $tRNA^{ser}$ from bakers' yeast (0.57 nmol serine A_{260} $unit^{-1}$) 10 mM $Mg(OAc)_2$, 1 mM DTT, 50 mM Bicine–KOH, *p*H 9.0, and enzyme. Incubations were carried out at 30 °C for 10 min and stopped by pipetting 40 μl of the reaction mixture on Whatman 3MM paper disks which were washed and counted[11].

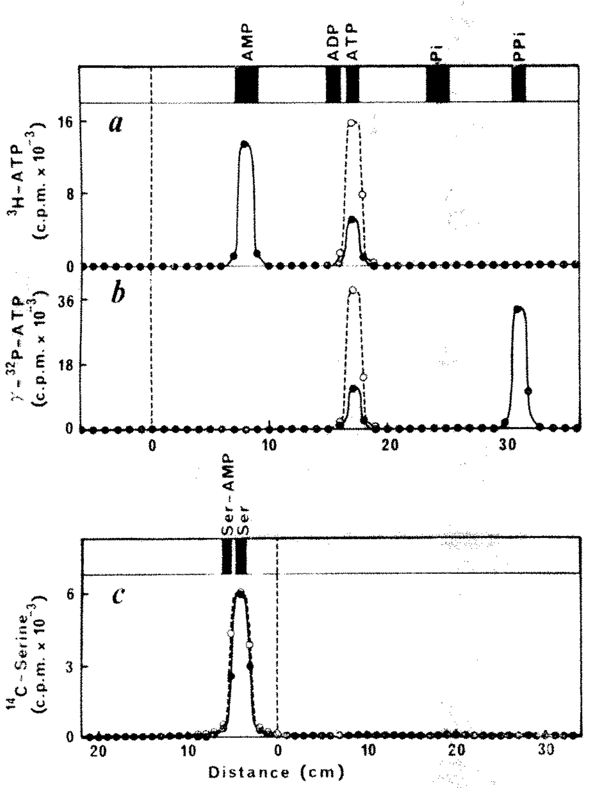


Fig. 1 Electrophoretic analysis of the reaction products of the 4HAQO-activating reaction. After the reaction electrophoresis was carried out on Whatman 3MM paper in 0.05 M sodium citrate, *p*H 3.9, at 37 V cm^{-1} for 90 min, the paper was cut and radioactivity was measured by liquid scintillation[4]. *a*, 2-^{3}H-ATP (0.2 mM; 125 Ci mol^{-1}; Radiochemical Centre, Amersham) was incubated at 30 °C for 15 min in the reaction mixture (0.1 ml) containing 10 mM L-serine, 1 mM dithiothreitol (DTT), 5 mM Mg(OAc)$_2$, 50 mM sodium borate, *p*H 9.0, 10 µg ml^{-1} enzyme in the presence (●) or absence (○) of 1 mM 4HAQO; *b*, γ-^{32}P-ATP (0.2 mM; 6.25 Ci mol^{-1}; Radiochemical Centre, Amersham) incubated as above *c*, U-^{14}C-L-Serine (0.01 mM; 130 Ci mol^{-1}; Daiichi, Tokyo); incubated in the reaction mixture in the presence (●) or absence (○) of 4HAQO as above except 2 mM ATP was added and unlabelled L-serine was omitted. Pi, Orthophosphate; PPi pyrophosphate.

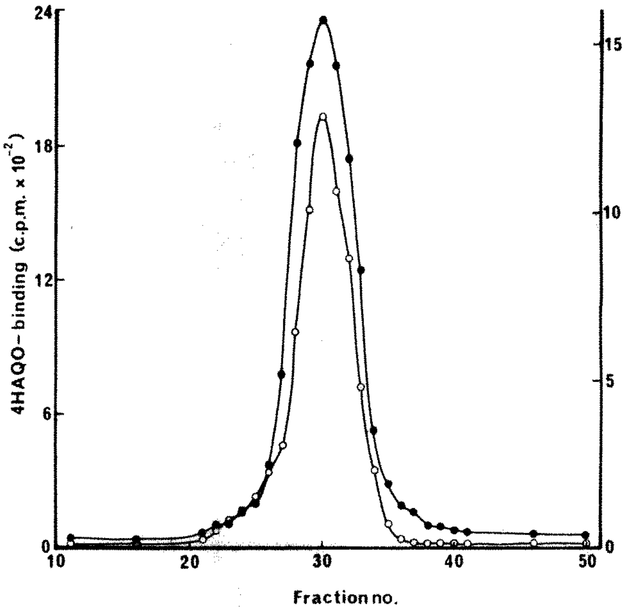


Fig. 2 Chromatographic elution pattern of 4HAQO binding (●) and seryl-tRNA-synthesising (○) activity from a Sephadex G-200 column (80 × 1.1 cm) loaded with 800 µg the purified enzyme, equilibrated and eluted with buffer C. The measurements of the enzyme activities were carried out as in Table 1.

same stability against heat inactivation. The specific activity of the purified enzyme for seryl-tRNA synthesis was comparable with that of highly purified yeast seryl-tRNA synthetase as reported by Heider *et al.*[11]. From these results we conclude that seryl-tRNA synthetase also catalyses the binding of 4HAQO to nucleic acid. Figure 3 summarises our proposed model for the enzyme-mediated reaction scheme of 4HAQO and nucleic acid. We assume, as is the case with other aminoacyl-tRNA synthetases (for example, ref. 12), that seryl-AMP is made on the seryl-tRNA synthetase molecule. As the addition of 4HAQO to this reaction mixture results in the consumption of ATP and the concomitant release of AMP and pyrophosphate, as in the case of the addition of tRNA (Fig. 1), we further assume that 4HAQO reacts with seryl-AMP bound on the enzyme, producing seryl-4HAQO, analogous to the way in which the addition of tRNA results in the formation of seryl-tRNA. This assumption is supported, though not proved, by the finding that the binding of 4HAQO to nucleic acid can be activated by seryl-AMP in the absence of the enzyme (Table 2). In this system, however, seryl-AMP hydrolyses so rapidly that the detailed kinetics of the reaction cannot be examined. Other kinds of aminoacyl-AMP, such as leucyl-AMP, also activate 4HAQO. The activated 4HAQO in the present system, either enzymic or non-enzymic, reacts with purines but not with pyrimidines giving rise to adducts. The activated 4HAQO is too unstable to be isolated and its structure determined.

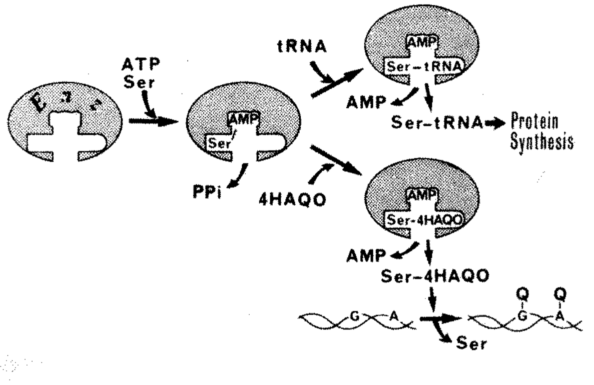
Table 2 Binding of 4HAQO to RNA by seryl-tRNA synthetase and by seryl-AMP

	Extent of binding (nmol 4HAQO)
Enzymic reaction	
Complete	3.54
Minus enzyme	0.09
Non-enzymic reaction	
Complete	6.55
Minus seryl-AMP	0.09

Enzymic 4HAQO binding was carried out in the reaction mixture as in Table 1 except that 2.5 mg ml^{-1} yeast RNA and 60 µg ml^{-1} purified enzyme were used. The reaction mixture (0.1 ml) for non-enzymic binding contained 1 mM ^{3}H-4HAQO (1.5 Ci mol^{-1}), 2.5 mg ml^{-1} yeast RNA, 1 mM DTT, 50 mM Bicine–KOH, *p*H 8.0, and 0.4 mM seryl-AMP which was synthesised according to the method of Berg[13]. Both reactions were carried out at 30 °C for 10 min and other conditions of assay were as described in Table 1.

It has been noted previously[9] that the 4HAQO-binding reaction including yeast preparation is stimulated only by L-serine, whereas the reaction with rat hepatoma cell prepara-

Fig. 3 A proposed mechanism of 4HAQO activation. In the first process of the reaction seryl-AMP (Ser-AMP) is formed on seryl-tRNA synthetase (Enz) from L-serine (Ser) and ATP. In the physiological pathway, tRNA then accepts the serine residue on the enzyme to form seryl-tRNA (Ser-tRNA) which is utilised in protein synthesis. If 4HAQO is present, however, it may be acylated by the seryl-AMP–enzyme complex. Seryl-4HAQO (Ser-4HAQO), a possible reactive species, may then introduce quinoline groups (Q) into nucleic acid at guanine (G) and adenine (A) residues (M.T. and M.T., unpublished).



tion is stimulated by both L-serine and L-proline. This suggests that 4HAQO may be activated by both seryl- and prolyl-tRNA synthetases in rat tissues. Aminoacyl-tRNA syntheteses which are capable of 4HAQO-activation, may have an unique conformation enabling them to aminoacylate the N-hydroxyl group of the carcinogen. Carcinogenic aromatic amines and nitro compounds undoubtedly require metabolic activation for their reactivity *in vivo*. The first activation step of these compounds seems to be formation of N-hydroxyderivatives, and it has been suggested that the final activation step is N-hydroxyesterification[14]. Seryl-tRNA synthetase and some other species of aminoacyl-tRNA synthetase may participate *in vivo* in the activation of not only 4HAQO but also some other N-hydroxy compounds through their aminoacylating capacity.

We thank Dr Y. Kawazoe for ^{3}H-4HAQO and discussions; Dr M. Miyazaki for purified tRNA; Professor S. Kondo for interest and advice; and Dr B. W. Glickman for reading the manuscript. This work was supported in part by grants from the Ministry of Education and from the Society for Promotion of Cancer Research, Japan.

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Received February 13; accepted April 17, 1975.

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K region benzo(α)pyrene-4,5-oxide is conjugated by homogeneous gluthathione S-transferases

WE report direct evidence that conjugation with glutathione (GSH) is a significant mechanism for detoxification of the epoxides of polycyclic aromatic hydrocarbons. We believe that the level of the enzymes may be directly relevant to the carcinogenic potential of the hydrocarbons, which are common pollutants[1]. They are metabolised by microsomal mixed function oxygenases and associated enzymes, to detoxified as well as carcinogenic forms[2-4]. One of the metabolic products of benzo(α)pyrene is the 'K' region epoxide, benzo(α)pyrene-4,5-oxide. Such epoxides bind to macromolecules, are mutagenic and may be the carcinogenic forms of the polycyclic aromatic hydrocarbons[5]. Benzo(α)pyrene-4,5-oxide is converted enzymatically either to the dihydrodiol by hydrolysis[6] or to a thioether by conjugation with GSH[7]. The glutathione S-transferases (EC 2.5.1.18) detoxify many compounds[8,9] including alkyl epoxides[10] and arene oxides[11-12]. Now that pure glutathione S-transferases are available (five rat[10,12,13] and five human, K. Kamisaka, W. H. H., J. N. K., I. M. Arias and W. B. J., unpublished), we have been able to evaluate directly the extent to which these enzymes can produce GSH conjugates of the polycyclic aromatic oxide and, presumably, detoxify it.

Incubation of each of the enzymes with benzo(α)pyrene-4,5-oxide and GSH in standard assay conditions[7] always resulted in the formation of the GSH conjugate (Table 1). Two other substrates of the enzymes are included for comparison: an alkyl epoxide, 1,2-epoxy-3-(*p*-nitrophenoxy)propane, and an aryl halide of pharmacological significance, bromosulphophthalein. Both compounds form the appropriate thioethers of GSH. Each of the purified enzymes was active in the conjugation of the polycyclic hydrocarbon epoxide with glutathione although relative activity varied considerably (Table 1). We stress that available information on the rat and human transferases (Kamisaka *et al.*, unpublished) suggests that these enzymes represent a large portion, 10% and 2%, respectively, of the total protein extractable from liver. Furthermore, one of the enzymes, transferase B[14], is identical to ligandin[15], a protein known to form a covalent derivative *in vivo* with 4-dimethylaminoazobenzene[16]. Knowledge of the role of the glutathione S-transferases should clarify our understanding of the routes of carcinogen activation and detoxification.

Table 1 Specific activity (nmol min^{-1} mg^{-1}) of homogeneous rat and human glutathione S-transferases

Enzyme		Benzo(α)pyrene-4,5-oxide*		Alkyl epoxide†	Bromosulphophthalein‡
		Exp. 1	Exp. 2		
Rat transferase	A	100	88	100	530
	B	14	11	0§	6
	C	127	99	0§	18
	E	51	70	6,100	0§
	AA	8	4	0§	4
Human transferase	β	25	—	0§	10
	δ	33	29	0§	1

*Incubation was for 2 min at 37 °C in 0.4 ml of 10 mM Tris-chloride, *p*H 7.5, 50 μM ^{3}H-benzo(α)pyrene 4,5-oxide in 15 μl of methanol and 0.5 mM GSH. After precipitation of protein with 0.5 ml acetone and centrifugation, the solution was subjected to thin-layer silica gel chromatography with *n*-butanol-*n*-propanol-2 M ammonium hydroxide (2:1:1). Radioactivity in the R_F 0.06 component was measured[7]. Two series of experiments were conducted with different amounts of the same enzyme. The values presented have been corrected for the small amount of conjugate, about 1 pmol min^{-1} mg^{-1}, produced in the absence of enzyme.

†Specific activity with 1,2 expoxy-3-(*p*-nitrophenoxy)propane as described before[10].

‡Determined spectrophotometrically as described before[12].

§A value of zero implies that nanomolar concentrations of enzyme did not produce conjugate in an amount greater than that of a control in the absence of enzyme.

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Received March 20; accepted April 23, 1973.

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reviews

Intelligent life in space

P. C. W. Davies

The Galactic Club: Intelligent Life in Outer Space. By Ronald N. Bracewell. Pp. 141. (Freeman: San Francisco and Reading, 1975.) Cloth £3.70; Paper £1.90.

Over the past decade there has appeared a number of books devoted to the subject of exobiology in general, and extraterrestrial technological communities in particular. The subject is exquisitely fascinating, provocative, and distinguished by a current lack of concrete observational data. These ingredients form a fertile situation for all sorts of unsound speculation so that it is particularly important to have available books on the subject written by competent scientists with established reputations.

One such individual is Ronald Bracewell, of Stanford University, who was among the first contributors to the emergence of scientific consideration of extraterrestrial 'intelligent' communities and the problems and possibilities of radio communication with them. In what Bracewell clearly regards as the pioneering work on the subject, Giuseppi Cocconi and Phillip Morrison suggested in 1959 that radio signals from a technical community in outer space might be directed at us at 1,420 MHz, a natural choice of frequency corresponding to the emission from hydrogen.

Following this proposal, Bracewell detailed three distinct cases for consideration: abundant life, with the nearest community within 30 light years; sparse life within say 30–300 light years; and rare life (beyond 300 light years). He pointed out an important connection between the distance of the nearest community, and the average lifetime of the community. If this lifetime is to be measured in many millions of years, then life in the galaxy may well be abundant.

These arguments, together with some technical considerations about radio telescopes and search patterns, are presented in a short volume called *The Galactic Club*, a term which refers to the expected network of information exchange between galactic communities. This is a well written and interesting book, with a rather unusual presentation. Many full page paintings and lithographs of a distinctive style punctuate the text, though their connection with the subject matter is occasionally obscure.

The book encompasses a rather odd conjunction of topics. After a brief introduction to the astronomical, chemical and biological basis of exobiology, Bracewell resurrects the Velikovsky affair recalling that author's fantastic speculations about Solar System cataclysms. Returning to the main theme, some discussion is presented on the exact procedure whereby mankind might intercept interstellar messages, the nature of the message and the response. A chapter is devoted to the ambitious and awesome scheme known as Project Cyclops which proposes a vast array of radio telescopes all busily skimming the sky for messages.

Another chapter is devoted to the remarkable idea of interstellar messenger probes. Based on the assumption that near-luminal space probes are unlikely to be a practicable possibility at any level of technological attainment, it is suggested that, rather than indulge in interstellar travel themselves, intelligent space people would send out small probes at modest speed, to lie in wait around likely looking stars in the hope of eventually contacting a nascent technical community. The strategy adopted by such an interloper would be to echo domestic radio messages, thereby attracting the attention of an assured audience. The idea is bound to provoke the thought: is there such a probe in the region of the Earth even now?

This type of discussion leads quite naturally to the question of whether the Earth has been (or still is) the recipient of extraterrestrial visitation. In this context, some of the highly popular and evidently fanciful claims of Erich Von Daniken are briefly mentioned, with the conclusion that they satisfy a deep sociological need and "represent a substitution of faith for reason".

Further spectacles are provided by an outline of Gerard O'Neill's amazing studies of spinning space cylinders. These monster stations, envisaged as many miles across, are one idea of a future space colony.

The Galactic Club will have a considerable appeal to popular readership, providing as it does an intellectual journey into a strange and wonderful world of cosmic speculation.

To the scientific community it presents an interesting background to astronomy and space science and, at a more fundamental level, serves to remind us all of a wider perspective of human society.

Dry dispersion

P. G. Williams

Dispersion Theory in High-Energy Physics. By N. M. Queen and G. Violini. Pp. xi+202. (Macmillan: London and Basingstoke, January 1975.) £12.00.

Dispersion relations have been a part of the armoury of high energy physics for nearly 20 years now. It may seem surprising that not very many monographs have been exclusively devoted to this subject over the years. I believe that even a casual reading of this latest offering from Drs Queen and Violini will provide a clue as to why that is; on its own the subject can be rather uninspiring, and the authors have not succeeded in showing otherwise. Indeed, the text is written in such a dry, sparse manner with little or no indication of the excitement inherent in fundamental particle physics that I would never dare recommend it as a first introduction to my graduate students! Having said this, I must say that the book is well organised, very carefully written and contains a useful combination of material not previously available in book form.

After a short introduction to scattering formalism the authors obtain dispersion relations from causality, studiously avoiding any complications. Then follow two chapters, one on the Mandelstam representation, the other a rather nice treatment of high energy bounds. Thereafter, we find a six-chapter catalogue of various types of dispersion relations (excluding partial wave dispersion relations by explicit choice) and their applications. This is relieved by a chapter on Regge theory. At least I had hoped it would be until I read it and found the subject dried out to its very bones by its rather formal treatment. The final chapter, on the analytic continuation techniques of Cutkosky and Deo, is rather useful.

I can't say I welcome this new addition to the literature: the essential parts of the book are better suited to a review article; sections added by way of introduction for students are formal and not at all well physically motivated. And it is all at the astronomic price of £12.00 for a mere 200 pages.

Shedding light

The Theory of Polarization Phenomena. By B. A. Robson. Pp. viii+119. (Clarendon : Oxford; Oxford University Press : London, January 1975.) £5.50.

THIS book is one in the series of monographs *Oxford Studies in Physics.* It comprises just over 100 pages and contains only 40 references in its bibliography. It is therefore very selective in its material and is likely to be narrow in its range of utility. On the other hand, although a vast number of experimental measurements have been obtained there is a paucity of coherent theoretical treatments, so any light (polarised or otherwise) that can be shed on the subject is welcome.

The content of the book is aimed at the gap between undergraduate treatments and research review articles, and it will undoubtedly have most value for new researchers in the field. Starting from a classical basis, the book develops both the Jones and Mueller calculi. The comparison between the two is most valuable. The generality of the Mueller calculus which uses observable quantities and does not rely on the electromagnetic theory of light makes for a powerful and pragmatic description but the Jones calculus has an appealing simplicity for application to coherent beams and polarised light.

Assuming a background knowledge of quantum theory and matrix mechanics, the author develops the quantum theory of polarisation and then gives separate sections on spin $\frac{1}{2}$ and spin 1 particles in the non-relativistic limit. These treatments will be of increasing value as polarised beams of deuterons and other ions become available. The generalisation to the cases of arbitrary spin and polarised targets is of intrinsic value in view of the advent of superconducting magnets. Further sections include the emission and absorption of electromagnetic radiation and an indication of how to include relativistic effects.

Although my overall impression is of a neat and concise presentation, the complete absence of any experimental considerations will perhaps limit the ultimate value of this book. The few diagrams are of vectorial representations or of energy level schemes which leave much of the physical, three-dimensional aspects of the subject to the imagination.

What is now needed is another monograph to explain the physical meaning of this erudite formalism.

Richard J. Griffiths

Animal ultrasound

Ultrasonic Communication by Animals. By Gillian Sales and David Pye. Pp. x+281. (Chapman and Hall: London, September 1974.) £4.95.

THERE are good physical reasons why the wavelengths, and inversely the frequencies, of sounds generated and received by animals should be related to the size of those animals. Thus, it is no surprise that some of the smaller mammals use ultrasound in communication in the same ways that larger animals use sounds audible to man. But in addition to that a new field is opened up because those shorter wavelengths allow a much stronger interaction with the environment. Some of the technical feats involved in the use of ultrasound are truly remarkable : in addition to the echo-locating behaviour of bats there are a whole variety of evolutionary adaptations such as the ultrasonically silent aerodynamics of the owl, the ingenious sound generating mechanisms of insects such as grasshoppers, crickets and some species of moth, and the recent and varied developments in moths to detect and avoid bats, newcomers on the evolutionary scene.

Bats themselves produce a wide variety of signals depending on species and circumstances. Undoubtedly, the details of these will intrigue the technically minded reader, and those less technically minded will find the introductory sections of the book useful in explaining ultrasound, its interactions with obstacles, and its detection. It is only fair to point out, however, that only half of the material will interest such a reader. The remainder is clearly intended for the biologist and behavioural scientist.

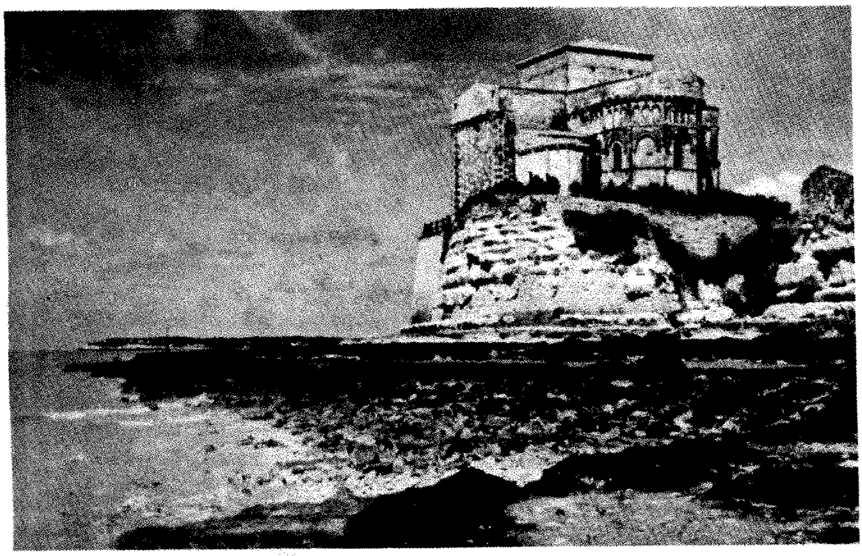
It might be argued that echo-location or navigation by sound is not communication and that the attempt to combine this with true communication leads to difficulties. It seems very arbitrary, for example, to limit discusson of communication between animals to a consideration of ultrasonic frequencies. Presumably, an integrated consideration of *all* sounds audible to the animals concerned, and comparison with other species not using ultrasound, would give much greater insight into the subject.

Such limitations are less obvious on the echo-location side as it is necessary to use the short wavelengths of ultrasound to detect small objects. In any case, the authors discuss examples of navigation using audible frequencies by the oil bird and cave swiftlet. One of the problems which faces the authors is the large number of species which have been researched, each with subtle variations in signals and behaviour, so that a comprehensive picture inevitably becomes repetitive.

The book naturally emphasises the authors' interests: bats, rodents, crickets and related insects. They devote much less attention to marine animals which indulge in reasonably sophisticated communication and rival in some respects the navigational abilities of bats. An area which stands out for future investigation is passive echo-location using ambient sounds which could in principle be much more effective at ultrasonic frequencies for small animals than for the blind man.

Generally, the photographic and line illustrations are adequate, but the oscillographs and sonograms have reproduced poorly and are not helped by the inartistic patterns of black masking. The book is well referenced and contains a wealth of information on animal signals that have only recently become accessible to man. Perhaps other readers like me will be tempted to construct bat detectors of their own.

J. P. Wilson



An 11th century church at Talmot in France. From *The Conservation of Cities.* Published as a contribution to European Architectural Heritage Year. Pp. 186. (Croom Helm: London; UNESCO: Paris, 1975.) £4.95.

nature

June 12, 1975

Behind the scenes at NAS

In May 1966, the Atomic Energy Commission received some sharp criticism from a committee of the National Academy of Sciences concerning its plans for getting rid of radioactive wastes. It wasn't the first time that the AEC had been stung by that particular committee and it was none too pleased. It promptly suppressed the report, informed the Academy that its advice on waste disposal was no longer needed, and said that its contract with the committee would be terminated the following year.

What did the Academy's leadership do to prevent the committee's expert advice on a matter of public importance from being ignored by an agency which didn't want to listen? It tacitly acquiesced in the suppression of the report by doing little to bring it to public attention, then it entered into negotiations with the AEC, as a result of which the offending committee was disbanded and the Academy appointed in its place a committee consisting almost entirely of former AEC officials and consultants. The AEC then renewed its contract with the more sympathetic panel.

That sordid episode is one of a number of skeletons in the cupboard of the National Academy of Sciences which are laid bare in a book published this week (*The Brain Bank of America*, by Philip M. Boffey, McGraw-Hill, $10.95) The fruit of an investigation begun in 1971 and supported by Ralph Nader's Center for the Study of Responsive Law, the book represents a remarkable blend of investigative reporting and careful scholarly analysis. It also shatters a few popular myths about the scrupulous objectivity of scientists called on for advice on matters of public policy.

Like many other learned societies, the Academy is a very prestigious body. Its members are elected for their outstanding contributions to scientific research and represent the élite in American science. Indeed, for the few dozen scientists who are accepted into the Academy each year, the honour is generally second only to being awarded the Nobel Prize.

But, unlike its counterparts in many other countries, the Academy is much more than just a scientific hall of fame. It attracted Nader's tender scrutiny because it provides massive amounts of advice, under contract chiefly to the federal government, on issues which touch the lives of virtually every American (and, indirectly, the lives of many people outside the United States as well). It operates literally hundreds of advisory committees concerned with topics as diverse as the safety of food additives, the need for new military hardware, the effects of SSTs on the ozone layer and the nutritional quality of dog food.

Established by an Act of Congress signed by President Abraham Lincoln in 1863, the Academy's government advisory work is performed through a body called the National Research Council (NRC), which is essentially the Academy's operating bureaucracy. (Two other organisations—the National Academy of Engineering and the Institute of Medicine—are also officially part of the Academy and operate through the NRC.) Briefly, when the Academy accepts a contract to undertake a study, the National Research Council reaches out into the scientific community and pulls together a committee of experts (who are not necessarily Academy members) which eventually turns in a report After review by a committee of Academy members, the report is passed on to the agency which asked for it.

Reports which bear the Academy's name tend to be influential documents because although they may be written by scientists who are not members of the Academy, they carry some of the Academy's considerable prestige. Moreover, because the Academy is an independent body with no particular axe of its own to grind, its advice is assumed to be untainted by the workings of vested interests.

But Nader was particularly concerned because the Academy had traditionally operated behind closed doors —a situation in which it is difficult for an outsider to judge how well it lives up to its reputation as an impartial adviser to the federal government.

Philip M. Boffey, a former reporter for the *Wall Street Journal* and *Science*, who conducted the investigation under Nader's sponsorship, has carefully peeled back the layers of secrecy surrounding the Academy's operations, leaving its biases and failings nakedly exposed. Rather than attempt to do a head count of all the skeletons he could find in the Academy's cupboards, Boffey applied his reporter's talents to seeking out the part the Academy plays in a number of important national issues involving science and technology. Certainly, it can be argued that his choices neglect some of the Academy's better works and Boffey himself acknowledges that the Academy has produced some reports of "outstanding quality", but Boffey's diggings have unearthed a number of flaws in the organisation's performances which seem to have broad implications.

Boffey's chief criticism is that the Academy is so dependent on the federal government for operating funds—it has a staff of more than 1,000 to support and the government provides some 80% of its budget—that it is too easy for government agencies to manipulate the Academy's committees. Specifically, Boffey suggests that there is sometimes a tendency for Academy committees to mute their criticisms in order not to lose future government contracts, agencies occasionally seek the Academy's support for decisions which have already effectively been taken, and the Academy is frequently asked to comment only on specific aspects of a policy rather than to examine the policy itself.

Boffey cites, and carefully documents, a number of examples of this "master-servant relationship" between government agencies and the Academy. The Academy's response to the AEC's threat to cut off funds for the committee on radioactive waste disposal is one such example. Another is the Academy's early role in the SST debate.

Although the Academy played no official part in the crucial decisions leading up to President Kennedy's 1963 announcement that building an SST would be a national goal, it was hastily brought in a year later when the government's sonic boom tests ran into public opposition. Asked to make a rather narrow study of the sonic boom problem, the Academy, according to Boffey, "showed a disquieting tendency to place itself on the FAA's (Federal Aviation Administration's) side of the controversy rather than act as an independent source of advice". An early Academy report even suggested that the government should launch a public relations effort to develop "attitudes sympathetic to the problem" among the general public. And a later report engendered so much controversy that 189 Academy members signed a petition asking for alleged inaccuracies in it to be corrected.

As for the charge that the Academy is sometimes used by government agencies to provide prestigious backing for policies which have already been decided, the Academy's role in the 1969 ban on the artificial sweetener, cyclamate, seems to be a case in point. When reports that cyclamates may be carcinogenic surfaced in October, 1969, the Food and Drug Administration was reluctant to act without the Academy's approval. With some hesitation, an Academy committee agreed to examine the relevant data at a meeting with FDA officials on October 17, and the next day Jesse Steinfeld, the Surgeon General, was able to announce at a press conference that "after a thorough review", the Academy has "independently confirmed our interpretation of the information and recommended that we remove cyclamates from the list of approved substances for general use".

Another important charge which Boffey makes is that the Academy is sometimes subject to corporate influences while it gives short shrift to the concerns of non-establishment scientists such as environmentalists and so-called public interest groups. He notes, for example, that the Academy committee responsible for advising on the safety of foods "has long and very close ties with the food and chemical industries", even to the extent that the food industry has financed its work, and some committee members were receiving research grants from the food industry while they were passing judgment on the safety of food additives. Boffey also documents the fact that key sections of a report on the hazards of airborne lead were drafted by an employee of a company which produces lead additives for gasoline, and he quotes statements by members of Academy committees advising on pesticides which are highly critical of environmentalists. Though he cites no instances in which committee members benefited directly from the advice they offered, Boffey suggests that he "found, surprisingly, that the Academy's advisory reports often fall short of the very high quality one would expect from the nation's preeminent scientific organisation; many, in fact, are mediocre or flawed by bias or subservience to the funding agencies".

But perhaps Boffey's most telling criticism is that, although the Academy is ideally placed to take the lead in debates on important national issues, it seldom does so. It was left to others, for example, to raise questions about the dangers of overuse of pesticides, to lead an investigation into the harmful effects of spraying millions of gallons of herbicides on Vietnam, and to examine such issues as nuclear reactor safety, automobile safety and the improvement of health care.

Boffey cites several reasons for the Academy's lack of leadership on such issues—including the fear that the Academy's reputation might be muddied if it gets involved in controversial political issues, and "reluctance on the part of some scientific leaders to oppose the government too vigorously lest they lose their potential ability to influence decisions." But he suggests that the chief reason is "undoubtedly the Academy's orientation toward serving rich and powerful institutions that do not generally encourage activism in behalf of reform". Although the Academy does get involved in important national issues, it frequently does so only at a late stage, and then only in response to specific requests for advice on narrowly defined topics, and it has "seldom, if ever, issued the kind of seminally significant document which revolutionises a nation's perception of public issues", Boffey contends. Another reason why the Academy rarely produces broad-gauged studies off its own bat is that it lacks free funds to do so.

What does the Academy leadership think of Boffey's criticisms? Academy President Philip Handler politely refused an interview to discuss the book on the grounds that he has had time only to skim the introduction and conclusion, and he is saving the rest for his summer reading. Nevertheless, in an address to the Academy membership in April, Handler called the book "a careful analysis of our defects" and he added that "the flaws which are detected by and large, it seems to me, are relatively minor, albeit real. And I think that we have erected arrangements such that most of those are unlikely to recur".

Handler has, indeed, instituted a number of reforms which should improve the Academy's operations. The NRC bureaucracy has been reorganised and streamlined, all reports issued in the Academy's name are now reviewed for accuracy by a committee of Academy members, committee members are screened for possible sources of bias, all unclassified Academy reports are now available for public inspection, and a small fund has been established to enable the Academy to launch its own studies on important topics.

But Boffey argues that those reforms are not sufficient because they "do not provide a fundamental solution to the Academy's major weaknesses: its servant-master relationship to the government agencies and industrial interests which provide financial support." He suggests that the Academy "should designate itself a national ombudsman . . and every Academy committee should approach its task as if it were representing the public rather than offering consulting services to a particular agency". Most important, it should scale down its activities to a level it can support from its own funds, accepting only those tasks which are deemed to be of great importance, demand the highest technical competence and require independence of judgment. It should also discard its belief that candour thrives only in secrecy, and throw open its operations to public scrutiny, and it should actively solicit ideas from activist groups, Boffey suggests.

Finally, Boffey notes in the preface that "if this book leaves one message with its readers, may it be the realisation that expert pronouncements must never be taken on faith They must be subjected to the same intense scrutiny and questioning as the public applies to political pronouncements This is no trivial matter, for the problems we highlight in this book are by no means unique to the National Academy of Sciences They exist in even more virulent form throughout thousands of organisations, advisory groups and institutions that affect our lives".

The myth of the objectivity of scientists on matters of public policy is indeed an enduring one —COLIN NORMAN.

international news

Register for UK radiation workers

from Peter Goodwin

THE UK's National Radiological Protection Board is setting up a comprehensive register of all UK radiation workers. Although radiation dose records are already kept by individual employers in the radiation industries, these data will, for the first time, be collected together in the register; epidemiological studies of radiation workers will then be much easier to carry out than they have been in the past.

The success of the register will depend on the cooperation of the different sectors of industry and of the UK Office of Population Census and Surveys (OPCS) which will play a vital part in tracing the medical histories and causes of death of those registered. Discussions are still in progress with the OPCS and with employers and trade unions, so final plans are not yet complete, but the NRPB is optimistic about the possibilities It hopes to be able to make a start within a few months by putting plutonium workers on the register, and then to follow this up by adding all other radiation workers to its files. The register will record internal exposure from ingested radioactive substances as well as external exposure.

A compelling reason for setting up such a register, according to the NRPB, is that it should help to establish the long term effects of low doses of radiation. Although there is every reason to believe, on the basis of experience in industries throughout the world which are involved with radiation, that the present permissible dose levels for radiation workers are safe, it has been difficult to establish this with certainty in the absence of a comprehensive record. Individual radiation workers move from job to job, and also take jobs in industries not concerned with radiation, so dose records are often incomplete. The permissible dose levels accepted at present are mostly calculated by extrapolation from studies made among such populations as the Japanese exposed to radiation from the Hiroshima and Nagasaki nuclear bombs and those exposed to radiation for medical purposes. But as such populations were subjected to far higher doses than are experienced in occupational situations, there is always some doubt as to whether the extrapolation is reliable.

The register is planned to be both retrospective and prospective. Retrospective epidemiological studies can be difficult to carry out because employers do not normally keep track of their employees after their employment has ended Also employees' National Health Service (NHS) Numbers are often lacking on the company records. A further complication arises from the 'notional dose' phenomenon: the practice of some companies of estimating employees' doses in the absence of genuine records. As 'notional doses' are generally high, to give a margin of safety, it is possible that retrospective studies can give the impression that a relatively high occupational dose is less harmful than it actually is. In spite of these difficulties, says the NRPB, the register should greatly facilitate retrospective investigations of possible radiation effects. Prospectively, it will ensure that all future dose records contain all relevant information, such as the employee's NHS number, so that his death certificate can ultimately be located, as well as date of birth, address, and other facts relevant to tracing his medical history. But as possible carcinogenic effects of low dose radiation are expected only to show up in the very long term, the prospective records are unlikely to be useful for assessing the safety of dose levels for many years, perhaps decades. ☐

THE vulnerability of the research teams attached to the smaller drug companies was made painfully obvious recently to the research staff of Aspro-Nicholas, the UK subsidiary of the Australian pharmaceutical concern The entire research staff of around 170 scientists and technicians at the Nicholas Research Institute at Slough were told that they had lost their jobs as the company had decided to close down its research effort completely. Although Nicholas does development work and has manufacturing interests in several countries, all basic new product research has been concentrated in the UK and will now cease. A small unit in Australia which carries out long-term toxicology studies will still remain in being.

Although possible cuts in the research budget and even a few redundancies had been envisaged by the staff as a result of the prevailing economic conditions, the wholesale closure of the laboratories came as a complete shock to those involved. The reasons given by the company were the rising costs of research and the decreasing chances of a significant breakthrough. About 120 of the staff have been given one month's notice and others have been invited to stay on for a few months to wind up projects.

The view that investment in pharmaceutical research is now yielding a steadily diminishing return is to some extent supported by surveys from the United States In 1959, 63 new drugs were put on the market there; in the early 1970s, in spite of a trebling in research funds, an average of only 15 new drugs each year appeared between 1971 and 1973.

This is small comfort to the staff of Nicholas, however, who felt that they had several promising lines of research which could have started to show a return during the next few years. The research effort at Nicholas has only been going at a significant level for about six years, a relatively short time in applied pharmaceutical research. Research was in progress into analgesics, anti-inflammatory agents, drugs affecting the central nervous system such as anti-depressants and minor tranquillisers, and cardiovascular drugs. Several compounds had reached the drug candidate stage. Nicholas was also evaluating several compounds initially developed in university laboratories.

A spokesman for the Association of the British Pharmaceutical Industry said that the association had been issuing warnings for some time that the high cost of research and the increase in time needed to bring a new product on to the market, together with a decline in profitability of the industry generally, has created a situation in which the research and development needed to produce new medicines is being placed in jeopardy.

Is Canada an energy-deficient nation?

from David Spurgeon, Ottawa

THE belief that Canada is rich in natural resources is generally accepted by Canadians as well as others, and is certainly widely held by their neighbours south of the border. It is, in fact, one basis of the USA's wish for joint control of North American resources.

It is thus interesting to hear from a professor of geology at Carleton University, F. Kenneth North, that this idea is largely illusory. "Canada," Dr North told a recent Conference on Technology and Canadian Foreign Relations at this university in Ottawa, "is the only energy-deficient nation in the world which believes itself to be energy-abundant and behaves accordingly."

Dr North has a habit of making flat, uncompromising statements, and he has been making quite a few of them regarding Canada's energy supplies in recent months. The kind of thing he told the Carleton conference was just what he has been telling federal authorities, although there is not much evidence that they have been listening.

Perhaps this is because they do not want to believe him. His message is that energy supplies—particularly oil—have been vastly overestimated and that, regardless of what action is taken, Canada faces a severe energy crisis before the end of the 1970s.

"No matter how carefully we establish our priorities, no matter how expeditiously that conversion to other energy forms can be brought about, no matter how successful our conservation measures may become, social and economic disruptions on a vast scale are inevitable", he says. "The United States will be a much less affluent and comfortable place than it has been. Canadians should be wondering whether large parts of their country might not have to be evacuated."

That is pretty strong stuff, and the recourse of some who hear it is simply to recoil into their natural optimism. Philip H. Tresize, another delegate to the Carleton conference (organised by the Norman Paterson School of International Affairs), did just that. Now a senior fellow at the Brookings Institute and a former US ambassador to the OECD, Mr Tresize replied: "I am not impressed with the idea we're running out of resources . . . I can't challenge Dr North, but the world is made up of little else than raw materials, and our history has been that we've consistently found new resources, and I have no reason to believe this will not continue to the end of time."

Dr North had more facts than that at his disposal. Canada has always been regarded by her American neighbours as "some kind of resource cornucopia." he said. "But Canada is not remarkably rich in primary resources in proportion to its size. The number of really large mines in Canada can be counted on the fingers of one hand, and we have no very large oil or gas fields. There are individual copper mines in Chile and Africa capable of producing more new copper than all Canadian mines put together. Many of the richest gold and silver camps are in the western Cordillera of the Americas.

"Though we have 7% of the Earth's land area, and more than 7% of its area of continental crust, we have less than 2% of its conventional oil," said Dr North. "Even our vast reservoirs of fresh water are easily misrepresented. Far too much of it lies on the surface in easily polluted lakes; for a land of Canada's size, we are short of good subsurface aquifers because so much of our territory is either Precambrian shield or impermeable igneous rock.

"Canada owes her outstanding mineral production to her geographic and economic relationship to the United States. We have allowed them to establish our country as their resource hinterland. Canada's gross mineral production constitutes 7% of our gross national product. Ninety per cent of it is exported. Of all Canadian exports, of all materials to all trading partners, nearly one-sixth is of minerals and fossil fuels to the United States."

Canada's total known remaining oil reserves represent less than 15 months of present North American consumption, Dr North said. The proven tar sands reserves represent for North America an extension of four years in the life of its reserves—not hundreds as many imagine. Canada's total known natural gas reserves, including those in the Arctic, which may never be made available, constitute a supply of about 30 months at present North American rates of consumption And the reserves of gas so far known in the Mackenzie Delta, after nine years of drilling, would keep the proposed 48-inch pipeline filled for 4½ years.

North's pessimism extends beyond Canada, however Outside the Communist world, the rate of finding oil has shown a perceptible decline since 1963, he says, and outside the Persian Gulf, the heyday of which is now past, the 'free-world' has found only four basins having recoverable reserves of oil large enough to satisfy a single year's global demand.

The only new basins capable of yielding prolifically are in remote or unreliable regions, chiefly offshore, and statistical probability studies show that the Earth's crust is "very unlikely to possess more than a handful of prolific basins still to be discovered. Even if each of these is presumed to be as rich as the Alberta basin, or the North Sea, we are looking at a quantity of new oil, from new regions, very much smaller than the quantity we have already produced and consumed."

This leads Dr North to predict that the level of world oil production will start to decline in about 1985, not halfway through the 21st century, as many authorities maintain.

Similarly, he says it is statistically unlikely that North America can possess more than one or two potentially prolific basins that are still undiscovered. Canada's rate of production must start to decline in a year or less, because reserves have already been declining for five years and the rate of addition to reserves has been declining for 10 years.

"Starting next year, our capacity to provide domestic oil will decline at an average rate of 110,000 barrels a day per year. Unless all demand growth can be curbed, therefore, even a new tar-sand plant every year would be incapable of maintaining Canadian self-sufficiency in oil. By 1981, our deficit will reach 1 million barrels a day . . . It is unlikely—almost inconceivable, in fact—that tar-sand production will reach even half that amount by then."

Such doom-saying did not impress Mr Tresize. If we run out of one resource, he said, human experience teaches us that we can have confidence we will find substitutes that will do just as well. ☐

A hundred years ago

DR FOREL, of Lausanne, has for several years been investigating what are known as the *Seiches* of the Lake of Geneva. *Seiche* is applied locally to certain oscillatory movements which are occasionally seen to occur on the surface of the lake. The phenomenon had been investigated by previous observers, among others by Saussure and Vaucher, who attributed the phenomenon to variations in atmospheric pressure; in this, Forel, who has most minutely investigated the phenomenon, agrees with them. The phenomenon is found to occur on other Swiss lakes, and Forel believes it will be found in all large bodies of water. Indeed, he recognises in the *Seiche* probably the most considerable and the grandest oscillatory movement which can be studied on the surface of the globe.

from *Nature*, **12**, 134; June 17, 1875

correspondence

Integrity in science

SIR,—In a leading article in *Nature* (September 29, 1972) there occurs the following passage relating to my then recently published book, *Science at the Crossroads:* "Professor Dingle goes on to complain that a promised leading article rounding off the correspondence has never appeared, apparently oblivious of the way in which his promises to 'bring discredit on the journal' may have discouraged the judicious summing-up for which he asked". Although this was misleading, I ignored it because my primary aim, as my book makes clear, was to ensure that respect for moral integrity should take precedence of all other considerations in scientific practice, and I did not wish to add the relatively trivial question of my own reputation to the already excessive number of possibilities that had been exploited to divert attention from that. On the one occasion on which I did defend myself, the reason was that that had become my only means of establishing beyond doubt that the statements in my book were true. I had then, as now, no object but to make known the actual degree of awareness of moral responsibility within the scientific community, with the ultimate aim of raising it.

I hoped that this feature of the situation had thus been finally disposed of. A recent incident now makes it necessary, however, for me to state the facts relating to the passage quoted above. I have a complete record of my correspondence with *Nature* on this matter, and the only possible basis for what is described as "his [Dingle's] promises to bring discredit on the journal [*Nature*]" is the following extract from my letter of May 20, 1969 to the then editor: "It is now about nine months since I sent you my reply to Synge: it remains unpublished, and it is becoming increasingly difficult to present *Nature's* attitude without reflecting discredit on the journal I repeat that I do not want to do this, but I must get the truth brought out, whatever it involves, and you are leaving me no alternative." There are many other passages in the correspondence in which I strongly urged the editor not to make necessary revelations which would lower the high reputation which *Nature* had acquired under the editorship of Lockyer and Gregory, which I had a strong desire to preserve, reinforced by my long and intimate association with the journal in Gregory's days. The reference to "my reply to Synge" is explained in my book; here it is necessary only to say that what is described as "the judicious summing-up for which he [Dingle] asked" was not asked for by me, but was spontaneously promised by the editor on November 24, 1969 (six months after my alleged "promises" which, it is stated, "may have discouraged" its appearance) as a substitute for my reply to Synge, which I would far rather have had and which might have settled the whole matter there and then (for details see my book). This, like the "judicious summing-up", has never been published.

This incident has implications for the future as well as, and far more important than, those for the past. Those who share my view of the supremacy of moral obligations over considerations of expediency in science can no longer remain unconvinced that the continued evasion or ignoring of the simple question concerning the rates of two clocks in uniform relative motion which I have asked many times—conspicuously in my book and lastly, so far as *Nature* is concerned, in my letter there of August 31, 1973 (hereinafter called L) —constitutes a violation of the basic ethical principle of science, with the gravest implications. If evidence of this were still needed, it would be given by the existence of a letter, signed by 12 persons highly qualified in the fields of physics, astronomy, space research, electrical engineering, the Open University, psychiatry, archaeology, religion (three separate branches of the Christian Church of world-wide repute), philosophy, law—who constitute a small fraction of the number who have expressed accordant convictions. This letter asks, in view of the objective facts disclosed in my book, for authoritative reassurance, by the provision of a direct answer to my question, that integrity is still preserved within the scientific community. It has not succeeded in being published in any journal where its appearance would be appropriate and effective. The signatories, many of whom claim no technical qualifications in relativity theory, see clearly that the question asked is perfectly intelligible without technical knowledge, and that the failure to provide an equally intelligible answer is unmistakably a moral failure.

Notwithstanding the obviousness of this to any reader of my book or of L, no comment on L of any kind has appeared in *Nature* until Kilmister's recent offer of a solution to the problem in the issue of December 6, 1974. In one of the *Three Musketeers* stories, General Monk's skill in diplomacy is said to be shown by his practice, when wished "Good morning", of not replying immediately but waiting twelve hours and then saying "Good evening". Kilmister seems to have emulated Monk. In L, in an attempt to prevent repetition of earlier evasions of my question, I repeated that it had nothing to do with, among other things, "coordinate systems or frames of reference". Kilmister waits 15 months and then says (*Nature*, **252**, 439; 1974) "The basis of Dingle's long-standing arguments with the relativists seems to be his insistence on the arbitrary nature of inertial frames." L is, however, still available for reference, and anyone may evaluate Kilmister's judgment by looking it up. He will see that there are only two possible ways of answering my question which are compatible with the ethical demands of science: they are (1) the completion by a single phrase of the following sentence: "The slower-working of *the two clocks, A and B, mentioned in the question*—which, as shown in L and elsewhere, the theory requires actually, and not merely apparently, to work at different rates—is that which . . ."; (2) acknowledgment that the theory permits no means of proving that A works more slowly than B that is not applicable with equal validity to prove that B works more slowly than A, and that, since this involves a physical impossibility, the theory is untenable.

I think it must now be clear to every reader, whether physicist or not, whose mind has not already been finally closed on this subject, that the basic issue here is a moral one, all intellectual, metaphysical and mathematical considerations being secondary, and that the possibility of continued belief in the moral integrity of the scientific community, the social significance of which in the present age needs no pointing out, depends on the early provision, by some body or person generally understood to be qualified to speak with authority on the theory, of one of these answers, unobscured by additional verbiage, diagrams or arrays of symbols of any kind.

HERBERT DINGLE

Purley, Surrey

SIR,—Your readers should first know the circumstances in which this exchange of correspondence is published. Professor Dingle has complained that the leading article of September 29, 1972, was defamatory of him, but has undertaken not to take legal action if you publish a letter (above) which I consider might be damaging of me, your predecessor, without the following explanation, however tedious.

I know of nobody who wishes to damage Dingle or his reputation I would be sorry, but at the same time exceedingly surprised, if the leading article to which he refers had been read in that sense. It was a forthright article but pallid compared with some of the polemics with which Dingle has entertained the readers of *Nature* in the past quarter of a century.

On one small point, whose relevance to his complaint is debatable, Dingle is right. His promise to "bring discredit" on *Nature* was made before and not after I rashly volunteered to write a leading article explaining why his argument is false. I abandoned this project after letters from him such as that of April 6, 1971, in which he wrote that "action on your part even now would make a full exposure of the ethical aspect of the matter unnecessary". It would no doubt have been more courteous to have told him explicitly that, as far as I was concerned, his dealings with *Nature* were at an end, but the correspondence had become offensive and repugnant. Dingle may not appreciate how the manner in which he has pressed his case has often forfeited him the indulgence that one of his age and wit would ordinarily command.

Your readers should know that the 'recent incident' referred to in Dingle's letter was my comment to the editor of an overseas journal that the author of an article submitted for publication and purporting to be an objective account of this business had at no time sought to obtain my side of the story

Dingle is wrong to claim that this is an ethical issue His view that special relativity is a house of cards has been widely aired and amply refuted. I note that he does not refer to the simple statement of the reasons why he is mistaken presented in the leading article of which he now complains.

Dingle's error is primitive, as can be told from his penultimate paragraph He says it is a "physical impossibility" that clock A should work more slowly than clock B and that the reciprocal should also be true. Let him measure time by the frequency of a laser, and suppose two identical lasers pointing at each other are in relative motion. he light received at each laser will be out of tune with the local standard and the phenomena observed at the two lasers will be identical. Knowing that the two lasers are identical, each observer can construct an algorithm so as to infer what time is being kept by the other and will rediscover the familiar and the relativistic Doppler correction In other words, each frequency comparison will show that the distant laser is "running slow". Dingle's assertion that this is a physical impossibility is tantamount to the assertion that it is physically impossible for the velocity of light to be independent of its direction.

Dingle's confusion stems from his assertion that special relativity requires that the differences of rate should "actually and not merely apparently" occur. The truth, of course, is quite the opposite The theory is cast in that positivist mould in which no meaning can be attached to physical quantities unless they are observed or made "apparent". It explicitly rejects the use of physical quantities which cannot be measured. By supposing that there can be measures of time more "actual" than those based on measurement, Dingle is simply asserting that he holds to the pre-relativity notion of absolute time.

Dingle likes to think of himself as the boy who first announced that the emperor had no clothes, but what he is really doing is to require that all innovations of physical theory should satisfy the test of commonsense. He cannot see that special relativity is important precisely because it modifies Newtonian commonsense In the twelfth century, I suspect he would have resisted the notion that the Earth is round in similar terms, by issuing a challenge that the round earthers should either complete the sentence "the bottom hemisphere (from which people will be decanted into nothingness) is that which . . ." or recant.

It may be asked why, if Dingle's question has such a simple answer, it has not been provided before the appearance of the leading article two and a half years ago The explanation is that Dingle has shifted his ground In 1968, he seemed to be seeking to demonstrate that special relativity is internally inconsistent Now he appears to accept that the theory is consistent, but says that its consequences are unacceptable

It is also fair to say that the issue, through no fault of Dingle's, has often been confused by debates about situations where the accurate definition of inertial frames is crucial but difficult.

In all the circumstances, if there is a moral obligation undischarged, it rests with Dingle. He should acknowledge his error, so inform his small, devoted and in my view misguided band of supporters and then finally make his peace with the relativists

JOHN MADDOX

London EC4

Scientific exchange

SIR,—A brief note by Wendy Barnaby in your issue of May 8 reports on a new exchange agreement between "Swedish and Russian Academies of Science" and comments that the "agreement is unique in that, unlike the others the Russians have with western countries, it allows for the host country to invite specific scientists by name for study visits rather than accepting the other country's nominations." This principle is extremely important in the normalisation of scientific relations with the Soviets, and I should therefore like to point out that the National Academy of Sciences of the USA sought and obtained such an "invitational" provision in its exchange agreement with the Academy of Sciences of the USSR in 1968 applicable to one-month lecture visits by outstanding scientists and broadly applicable to all categories of exchange scientists, including those spending up to a year at research, in our Inter-Academy Exchange Agreement for 1974 and 1975.

Although this invitational approach seemed to be alien to the Soviet Academy's desires and practice with regard to a bilateral exchange agreement, the USSR Academy has increasingly acceded to the wishes of the National Academy of Sciences when it has requested consideration of the inclusion of individual named Soviet scientists whose presence is particularly desired at American universities.

LAWRENCE C. MITCHELL

National Academy of Sciences,
Washington 20418

From him and her

SIR,—We think he doth edit with ghastful grammar: "A year's free subscription for he or she . . Ed." (May 8)

Egad!

EDWARD B. ARMSTRONG
ELIZABETH B ARMSTRONG

Narrabri, NSW, Australia

Oh woe. Even Fowler, often a friend in need, allows me no escape from this rebuke save (a) that "it is hard not to sympathise with the victims of this trap", and (b) that Dickens did it, once But the competition, in spite of all, is still open. ED.

news and views

Measuring the effects of ghosts

from W. T. Toner

THE only statement about relativistic quantum electrodynamics which has consistently been made ever since 1927, when Dirac attempted to frame a modern theory of the motion of charged particles, is that it must be wrong and will break down. It is also the only feature of QED not yet verified.

At first, the doubts were because the ideas were spectacularly bizarre. Antimatter was predicted at a time when the only known particles were the nucleus and the electron, and empty space became filled with the ghosts of uncreated particles of all energies. The diagrams developed by Feynman into a powerful tool to deal with the extremely complicated algebra of the theory enable one to visualise the kinds of process it calculates. A few of the infinite set which describe the effects of an external field on an electron (or muon) are shown in the figure, where the observable objects are the external field (×) and the particle as it enters and leaves the interaction region. All other particles (lines) and propagating fields (wavy lines) are evanescent. Only the sum of all terms can be measured. Fig. 1*a* represents the force experienced by a classical particle. In *b*, the particle emits a 'virtual' photon before the external interaction and reabsorbs it later, and in *c* the virtual photon turns for a short time into a ghost electron–positron pair. The effects of all strongly interacting particles from pi-mesons to the new ψ-particles are lumped together in *d*, which needs electron–positron annihilation data for its evaluation, in the absence of a theory of the strong interaction. The higher order terms such as *e* are so complex that the algebra as well as the arithmetic is done by computer.

In each order, it is necessary to remove the infinities which accompany the mass and charge of the idealised particles of the theory in a self consistent way, so that the mass and charge which appear in the final expression are the values which an experiment would measure. The solution of the formidable technical difficulties of this procedure does not lessen the unease that infinite subtractions create, although the recent discovery that other theories may also be renormalisable and QED not just a unique accident of nature gives one more confidence (see John Taylor in *Nature*, **254**, 560; 1975).

QED may not be philosophically or mathematically respectable, but does it work? In parallel with an effort to which almost every notable twentieth century theorist has made a contribution there has been a determined attack by the experimentalists.

The first question is, do the processes actually take place at roughly the right rate? Most of the answers—all yes—have been given years ago but some have had to wait for the present generation of high energy accelerators. Frequently, the initial results have been contradictory. A recent example concerns the production of pairs of electrons and positrons by a heavy particle moving in the coulomb field of a nucleus. Jain *et al.* (*Phys. Rev. Lett.*, **32**, 1460; 1974) claimed an anomalous result for pair production by muons, and are now contradicted by Fortney *et al.* (*Phys. Rev. Lett.*, **34**, 907; 1975) who use a much more powerful and unambiguous technique to show that the theory works well in the related case of electromagnetic pair production by pi-mesons. (Not to be confused with production through strong interactions, which has a quite different behaviour.) It pays to choose the right technique.

In violent collisions at high energies, the dominant contribution often comes from one diagram which can then be tested under extreme conditions. Some of the results from the SPEAR storage ring which tend to be overlooked in the excitement over the ψ particle are tests of the point-like behaviour of the idealised QED electron to distances of the order of 7×10^{-16} cm. It is noteworthy that satellite measurements of the Earth's magnetic field confirm that the inverse square law holds to distances of $\sim10^{10}$ cm. There must be something right about a theory which spans such a range.

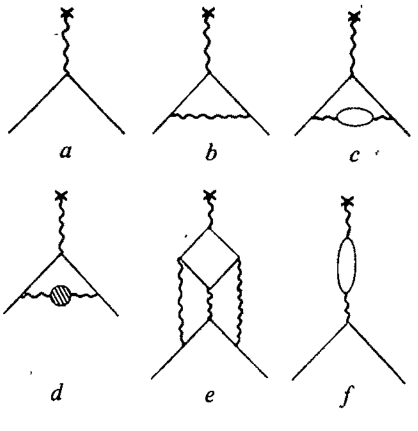


Fig. 1 Some of the diagrams representing the interaction of a particle with a field.

Our knowledge of higher order terms continues to improve. Bailey *et al.* (*Phys. Lett.*, **55B**, 420; 1975) in the third of a beautiful series of experiments begun fifteen years ago at CERN have been able to increase the sensitivity of their measurements of the non-classical part of the magnetic moment of the muon to the point where the effects of Fig. 1*d* can be seen. Their result is $(g-2)/2=(1,165,895\pm27)\times10^{-9}$, where g is the Landé g-factor, equal to 2 for a classical muon. Excellent agreement with theory is obtained if Fig. 1*d*, $(73\pm10)\times10^{-9}$, is included.

A second virtuoso experiment described in the same issue of *Phys. Lett.*, (**55B**, 411; 1975) by Bertin *et al.* is a measurement of the splitting between the 2s and 2p levels in helium ions where one muon has replaced the two electrons. Muons produced at the 600 MeV synchrocyclotron at CERN are brought to rest in a container of helium gas, surrounded by counters.

In a small fraction of the cases, the muons become trapped in the 2s level. On the signal that a muon has stopped in the gas, a Q-switched ruby laser is triggered. It in turn generates a brief pulse of infrared radiation from a tuneable dye laser. If the wavelength is just right, a $2s_{1/2}$ muon will be raised to the $2p_{3/2}$ level and will promptly drop down to the 1s ground state, emitting a tell-tale 8 kilovolt X ray as it goes. The laser was slowly scanned from 8,090 Å to 8,180 Å during the course of the experiment. The signal was contained in just 77 events in a narrow peak at 8,117 Å, out of nearly a million triggers.

Again, excellent agreement with theory is obtained, in this case giving the first precision measurement of the effect of Fig. 1*f* in atomic physics.

There is a small discrepancy with theory in muonic atoms where the nucleus is lead rather than helium. The theoretical difficulties now have a different aspect, as the power series is developed in Ze^2 rather than e^2. Chen (*Phys. Rev. Lett.*, **34**, 341; 1974), now supported by Papatzakos (unpublished) claims to have resolved this discrepancy by including a previously neglected term. Others (*Phys. Rev. Lett.*, **34**, 399; 1974) disagree.

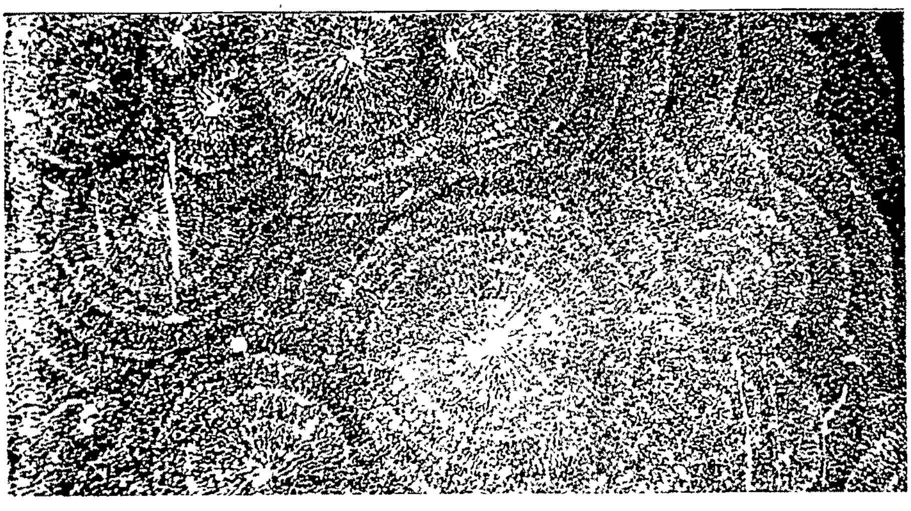
When do we stop? Two fundamental questions go completely unanswered as the theory now stands: Is it really infinite? Where did the muon come from? The key to both these questions may lie in the other interactions—weak, or even gravity. Some of the urgency behind the advocacy of the next generation of storage rings such as EPIC is due to the fact that weak interaction effects in QED processes should be clearly measurable with these machines.

Periodic cyclic AMP signals and cell differentiation

from J. D. Gross

THE cellular slime moulds, of which *Dictyostelium discoideum* is the best studied species, multiply as isolated individuals. When deprived of food they embark on the remarkable developmental phase of their life cycle. A few hours after starvation the cells begin to aggregate to a number of collecting points to form organised multicellular structures which ultimately become transformed into fruiting bodies made up of a cluster of spores held aloft by a thin column of vacuolated stalk cells. Two important observations on the events leading up to aggregation are reported in this issue of *Nature*. Shaffer (page 549) shows that cells exposed to a whiff of cyclic AMP themselves secrete cyclic AMP. This result puts the final seal on the evidence that cyclic AMP is the chemotactic signal by which the cells communicate during aggregation. Gerisch *et al.* (page 547) present evidence that these cyclic AMP signals are not only used to orient the amoebae but also play an important part in directing the physiological transformation of the cells from the actively multiplying state to aggregation competence.

During the period of interphase between the onset of starvation and the appearance of clearly visible aggregation patterns the cells show an increase in responsiveness to cyclic AMP in chemotaxis tests such as those devised by Konijn and by Bonner (Bonner *et al.*, *Devl Biol.*, **20**, 72; 1969). Fully responsive cells have been shown to possess a potent surface-located phosphodiesterase that hydrolyses cyclic AMP, as well as a protein that binds cyclic AMP and is thought to be the receptor for the chemotactic response (Malchow and Gerisch, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2423; 1974). The cells can synthesise and secrete pulses of cyclic AMP in response to a signal, and some, the pacemakers for aggregation, actually secrete cyclic AMP spontaneously in a pulsatile fashion. The cells also display new cohesive properties associated with the appearance of sites (contact sites A) that permit the establishment of strong end-to-end contacts between cells (Beug *et al.*, *J. Cell Biol.*, **56**, 647; 1973). Once aggregation is under way waves of inward movement travel out from the pacemaker cells each time they release a pulse of cyclic AMP (see figure), and these waves of inward-moving cells gradually become transformed into radial cell chains or 'streams' moving towards the centres. Evidently cells within range of a sufficient concentration of cyclic AMP respond not only by an inward movement step but also by relaying the signal. The outward propagation of the wave of movement requires that cells be refractory for some time after stimulation in order not to respond to the signal released by the cells distal to them. It is the central idea of a relayed pulsatile signal, first proposed by Shaffer (*Am. Nat.*, **91**, 19; 1957) that is firmly established by his latest results, as well as by similar observations of Roos *et al.* (*FEBS Lett.*, **53**, 139; 1975).



Waves of chemotactic movement in aggregating cells of *D. discoideum* NC4, prepared by the method of Alcantara and Monk (*J. gen. Microbiol.*, **85**, 321; 1974). Most of the well-developed territories have spiral centres (see Durston, *J. theoret. Biol.*, **42**, 483). The photograph is approximately twice life size and was taken by M. Peacey and D. Trevan.

Work in several laboratories is now beginning to indicate that the various properties of aggregating cells do not develop autonomously but rather depend on interaction between the cells and the signals they emit. The evidence in this direction now obtained by Gerisch *et al.* is of two kinds. First, they find that if cells are permitted to enter stationary phase of growth rather than being taken at the exponential phase they fail to aggregate and that this developmental defect can be overcome by pulsing the cells with cyclic AMP for several hours to stimulate natural signalling. Second, they find that the time taken for 'normal' cells to reach the aggregation stage is decreased somewhat by this treatment whereas it is delayed substantially by applying cyclic AMP continuously. The latter treatment would be expected to interfere with pulsatile signalling set up spontaneously by the cells.

Darmon, Brachet and Pereira da Silva (*Proc. natn. Acad. Sci. U.S.A.*, in the press) have observed a similar acceleration of the development of normal cells by pulsing, as well as correction of the aggregation defect in certain mutants by the same treatment. A rather different approach has been taken by Alcantara, Bazill and Monk (unpublished) who analysed the observation that the time taken for cells to develop aggregation-competence increases markedly with decrease in cel density (Alcantara and Monk, *J. gen. Microbiol.*, **85**, 321; 1974), and found that the effect of low cell density could be overcome by the addition of purified cyclic AMP phosphodiesterase. This enzyme, by rapidly destroying extracellular cyclic AMP, presumably permits pulsatile signalling to be established at a time when the total activity of the enzyme in the dilute cell suspensions would otherwise be insufficient.

At first sight all these results seem to lead to the paradoxical conclusion tha signalling between cells is itself required for the development of the ability to signal. This problem can be overcome by assuming that actively multiplying cells possess a weak capacity to detect and relay cyclic AMP signals, or develop this capacity during the first few hours of starvation in the absence of external signals, and that the ability to signal spontaneously only arises later (Robertson *et al.*, *Science*, **175**, 333; 1972). The first cells to emit cyclic AMP are apparently incapable of sustained signalling (Durston, *Devl Biol.*, **37**, 225; 1974). But their spasmodic signals could be relayed over large distances, leading to increased responsiveness of the cells and to the gradual emergence of pacemakers able to emit regular signals. It is not yet known how the build-up of the components of the signalling system and of the cell contact mechanism is coupled to the signal response system, nor whether it involves an effect on gene expression. Coupling may occur by way of the intracellular oscillations of cyclic AMP levels (Goldbeter, *Nature*, **253**, 540, 1975) that probably accompany both spontaneous and induced signalling

Control of ribosome synthesis

from A. A. Travers

A MAJOR activity of the bacterial cell is the synthesis of ribosomes. During normal exponential growth this process is finely regulated so that the rate of production of ribosomal RNA to a first approximation balances that of ribosomal proteins. Under these conditions the net synthesis of the principal macromolecular components of the ribosome is thus coordinated.

Another facet of the regulation of ribosome biosynthesis is the stringent response. When a stringent (*rel*$^+$) strain is functionally starved of an amino acid the extent of rRNA and tRNA synthesis is immediately curtailed. In contrast relaxed (*rel*$^-$) strains do not restrict stable RNA synthesis on amino acid deprivation. Another very early and apparently invariant manifestation of *rel*$^+$ gene function during the stringent response is the accumulation of the guanine nucleotide ppGpp, itself a metabolic product of the ribosome. Its discoverers, Cashel and Gallant, proposed that this unusual nucleotide might act as a direct inhibitor of rRNA (and tRNA) synthesis. Consistent with this hypothesis is the observation that under conditions of partial amino acid starvation in a *rel*$^+$ cell the rate of rRNA production is inversely correlated with the intracellular ppGpp concentration (Fiil, von Meyenburg and Friesen, *J molec. Biol.*, **71**, 769; 1972).

Is the coordinate control of rRNA and r-protein synthesis that operates during normal growth maintained during the stringent response? Two recent complementary studies have produced strong evidence that to a large extent it is. Bennett and Maaloe (*J. molec. Biol.*, **90**, 541; 1974) showed that like rRNA synthesis, the rate of r-protein synthesis relative to total protein synthesis is inversely correlated with the intracellular level of ppGpp. Similarly Dennis and Nomura (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 3819; 1974) found that partial amino acid starvation resulted in the synthesis of rRNA and r-protein being more strongly inhibited than total protein synthesis. These same workers have now confirmed (*Nature*, **255**, 460; 1975) that this stringent control of r-protein synthesis reflects the regulation of the synthesis of mRNA coding for r-proteins. Thus as with rRNA synthesis stringency operates here at the level of transcription.

r-protein mRNA is the first class of messenger whose synthesis has been directely demonstrated to be under stringent control. Its regulation contrasts with that of φ80, *lac* and *trp* mRNA whose production is not inhibited in an amino acid starved *rel*$^+$ cell The simplest model for stringent control is thus to consider the synthesis of one class of mRNA being regulated in concert with rRNA and tRNA while the synthesis of a second class of mRNA is relatively unaffected Any other mRNA species that are wholly or partially subject to stringency remain to be identified.

Although stringency is an important mode of regulating ribosome biosynthesis Dennis and Nomura hint that other controls may be superimposed on this rather coarse control because different ribosomal proteins are synthesised in vastly disproportionate amounts when the amino acid starvation becomes more severe. The authors suggest that this perhaps reflects an autogenous control of r-protein production.

Eukaryote genes in *Escherichia coli*

from David Sherratt

SEQUENCE-SPECIFIC restriction endonucleases are now well established tools for inserting both eukaryote and prokaryote genes into plasmids, thus allowing gene organisation and expression to be analysed. The endonuclease *eco*RI cleaves DNA about once every five to ten genes, generating short, self-complementary, cohesive ends. A number of plasmids and derivatives of bacteriophage λ contain a single *eco*RI site; as they can still replicate in *E coli* after other genes have been inserted at this site they provide suitable 'molecular vehicles' for cloning of inserted DNA sequences. The molecular vehicle is added to the required DNA and the mixture is cleaved with *eco*RI, annealed, sealed with ligase and used to transform *E. coli* (or in the case of λ, to transfect *E. coli*). With plasmid vehicles, plasmid-borne drug resistance is usually used to select transformants and these are then analysed for the presence and nature of inserted DNA.

Clearly then, in order to isolate hybrids (or 'chimaeras') containing specific inserted sequences, one needs either a source of DNA highly enriched in those sequences, or alternatively a means of selecting or identifying the few bacteria containing the required genes from within a heterogeneous bacterial population. For the first approach, the amplified ribosomal genes of *Xenopus laevis* provide an ideal source of specific DNA which can be separated from the bulk DNA by its distinct buoyant density. It is therefore not surprising that this DNA was used in the work that led to the first report of replication and transcription of eukaryote genes in *E. coli* (Morrow *et al.*, *Proc. natn. Acad Sci. U S A.*, **71**, 1743–1747; 1974). Another way of isolating specific eukaryote genes is to use the fact that some eukaryote mRNAs are relatively stable and are synthesised in large amounts by particular cells. Purification of these mRNA species followed by use of reverse transcriptase and DNA polymerase should allow synthesis of the relevant gene. Cohesive ends could be added *in vitro* using deoxynucleotidyl terminal transferase (see, for example, Jackson *et al.*, *Proc. natn Acad. Sci. U.S.A.*, **59**, 2904–2909, 1972). Possible candidates for such synthesis are globin, immunoglobulin and crystallin genes. Another possibility for small genes, for example, those specifying polypeptide hormones, is complete *in vitro* synthesis of a nucleic acid that should specify the known amino acid sequence.

Using the alternative approach, Kedes *et al* (in this issue of *Nature*, page 533) have now described an elegant method for the identification of bacterial clones containing specific DNA sequences from within a heterogeneous bacterial population transformed with a mixture of *eco*R1-cleaved plasmid and unfractionated eukaryote DNA. This method is potentially applicable to the isolation of bacterial clones containing any particular DNA sequences for which a specific labelled mRNA (or DNA) 'probe' is available; transcription of the inserted DNA in *E. coli* is not necessary. Kedes *et al.* suggest that this method should be able to identify clones containing genes which occur at frequencies as low as 10^{-6}, although their experiments describe the isolation of bacterial clones containing sea urchin histone genes which represent about 0.25% of total sea urchin DNA and which provide the template for large amounts of histone mRNA synthesis during embryonic cleavage.

Unfractionated sea urchin DNA was mixed with plasmid DNA (5: 1 ratio) and the mixture was *eco*R1-cleaved, annealed, ligated and used to transform *E. coli.* About 5×10^{-4} of the plasmid-containing transformants were calculated to contain the histone genes and these were selectively enriched by an adaptation of the classical clonal enrichment technique of Cavalli-Sforza and Lederberg (*Genetics,* **41,** 367–381; 1956). Multiple samples (48, each containing 10^4 cells) were taken from the heterogeneous transformed population and serial three-fold dilutions of each were grown to stationary phase. Aliquots of all 48 samples of each dilution were pooled and assayed for the presence of histone genes by hybridisation to the labelled mRNA. The greatest dilution giving positive hybridisation was found; it should contain at least one and at most a few samples with cells containing histone genes. To identify these positive samples in 14 rather than 48 hybridisation assays, the 48 samples of this 'limit' dilution were arranged into a 6×8 grid and aliquots from every sample in each row were pooled and tested for their ability to hybridise to histone mRNA, as were aliquots from the samples down each column. In this way, the 'intercept' sample(s) was identified and the cells containing histone gene isolated from it by again arranging 96 cultures from single colonies into a 12×8 grid and identifying the intercept samples. By this technique, three independent clones containing histone sequences were isolated, each occurring at a frequency of about 5×10^{-4}, yet identified in about 40 hybridisation assays.

The hybrid plasmids from these bacterial clones containing histone genes were analysed in some detail. Most, if not all, of the sequences present in histone mRNA were present in two non-homologous *eco*R1 fragments having a total size of six kilobases (kb), the coding capacity of which is more than sufficient to specify the structure of the histone proteins. Kedes *et al.* propose that it is this six-kilobase unit that is repeated some 470 times to give the known complement of histone genes in sea urchins. That this histone DNA is transcribed in *E. coli* minicells was also shown though the authors fail to enlighten us on the intriguing question of whether these genes are also translated in *E. coli.*

Kedes *et al.* point out that the possibility of isolating and purifying groups of genes that function concurrently at different stages of development now becomes a reality. It must be remembered, though, that considerable amounts of eukaryote DNA may never be transcribed and so cannot be identified by a mRNA probe. Nevertheless, this type of experiment, along with that described by Wensink *et al.* (*Cell,* **3,** 315–325; 1974), in which a number of individually cloned random segments of *Drosophila melanogaster* DNA were both analysed and mapped by *in situ* hybridisation should be invaluable in elucidating the nature of the eukaryote gene.

How mating algae agglutinate

from a Correspondent

CONTACTS between cells are important in such diverse phenomena as egg–sperm recognition, embryogenesis and tissue reorganisation. The complementary sites on cell surfaces that are recognised by other cells are being studied in a variety of systems including mammalian embryos and sponges. In many ways however there is a lot to be said for choosing simpler systems such as *Chlamydomonas* or yeast, the genetics of which can be handled conveniently. Crandall *et al.* (*Proc. natn. Acad. Sci. U.S.A.,* **71,** 26–29; 1974) have demonstrated the usefulness of the yeast *Hansenula wingei* in this regard. Intercellular recognition of opposite mating types leads to strong agglutination. The sex agglutinins are components of the cell wall of the yeast in the haploid state and can be solubilised in active form by proteolysis They are mannan–protein complexes of widely heterodisperse molecular weight (15,000 to 10^8) and carbohydrate content (50–96%) whose synthesis is normally repressed in the nonagglutinative diploid state. The molecular basis of the strong interaction between complementary sex agglutinins of haploid cells of different mating types is unknown although both protein–carbohydrate and carbohydrate–carbohydrate complex formation could be involved since no agglutinin fraction has been shown to be quite free of residual protein.

The *Chlamydomonas* system has been exploited in similar fashion. *Chlamydomonas* is a unicellular flagellated green alga that differentiates in the vegatative phase into two mating types. These gametes are morphologically indistinguishable from undifferentiated vegatative cells or from each other. Nevertheless complementary surface sites are present since fully differentiated gametes from two mating types agglutinate when mixed, whereas homologous gametes do not. It is known that *Chlamydomonas* aggregation factors are glycoproteins present on the flagellar tips of the gametes. Glycoproteins isolated from gametes of one mating type can induce agglutination of the opposite mating type gametes. Homologous gametes or vegatative cells are not agglutinated. In other words during the process of gametogenesis the vegatative cells acquire at their cell surface glycoproteins that function in mating type specificity.

On the basis of sensitivity to purified enzymes of the agglutinative activity of gametic glycoproteins, Wiese and Haywood (*Am. J. Bot.,* **59,** 530–536; 1972) suggested that the active site of (+) gametes includes a sugar sequence and this sequence is recognised by protein(s) (perhaps also carrying a carbohydrate moiety) present in (−) gametes. The glycoproteins specific for mating type seem to be made *de novo* during gametogenesis and are rapidly (within an hour) renewed if they are artificially removed from the cells by trypsin. It is possible that extensive reorganisation of the cell surface occurs during gametogenesis. For instance simultaneously with the appearance of sex agglutinins, the gametes become agglutinable with concanavalin A (McLean and Brown, *Devl Biol.,* **36,** 279–285; 1974), whereas vegetative cells are poorly agglutinated. It is not clear whether the Con A binding glycoproteins are the same as the sex factors although McLean and Brown suggest that this is in fact the case. It is likely however that the Con A receptor sites (presumably mannose residues) are not involved alone in gamete recognition since simple sugars which inhibit Con A-induced agglutination do not affect intergametic agglutination. Of course it is still possible that gametic recognition requires an extended sugar sequence including mannose in the sex glycoproteins which, unlike the simpler binding requirement of Con A, is not competed for by simple sugars.

Some progress has been made recently on the protein component of the *Chlamydomonas* recognition sys- present in (−) gametes McLean and Bosmann (*Proc. natn. Acad. Sci. U.S.A.,* **72,** 310–313, 1975) have considered the earlier suggestion of Roseman (*Chem. Phys. Lipids*, **5**, 270–297; 1971) that recognition of cell surface sugar sequences may involve surface-located glycosyl transferases. These enzymes normally carry out reactions using oligosaccharide sequences as acceptors. The basic idea is that adhesiveness between cells is achieved by formation of an abortive or non-productive enzyme–substrate complex.

What McLean and Bosmann have done is to assess the ability of intact gametes of either mating type to utilise sugar nucleotide precursors in glycosyl transfer. It is argued that since the nucleotides do not permeate intact and viable cells, any transfer must involve participation by enzymes present at the cell surface. A wide variety of sugar transfer reactions involving galactose, mannose, N-acetylglucosamine, sialic acid, glucose and fucose were detected on *Chlamydomonas* gametes in this way. Transfer takes place on to endogenous glycoproteins presumably also located at the surface of the same or adjacent cells in positions accessible to the enzymes. McLean and Bosmann suggest that the glycosyl transferases and acceptors may be a contributing mechanism in gametic recognition and adhesion in *Chlamydomonas*. Thus, the sugar transfer reactions are enhanced several fold when gametes of opposite mating type are mixed. This interesting result indicates that one or both of the components of the putative recognition system (glycosyl transferase and its glycoprotein acceptor) are present in limited amount at the surface of gametes of either mating type and maximal complex formation involves adjacent cells of opposite mating type. It is assumed of course that such experiments measure surface-located reactions rather than simply reflecting damage to the cells by the conditions of incubation and the revealing of intracellular biosynthetic sites. Though this assumption is still unproven the preliminary results encourage the further use of the *Chlamydomonas* sexual system to define more precisely the recognition mechanisms operating between cells.

Lunar and terrestrial fields

from Peter J. Smith

It is far more difficult to obtain reliable ancient field intensities from rocks than it is to determine valid palaeomagnetic directions. Yet although the study of directions has proved to be the more fruitful on Earth, in the case of the Moon constraints on origin and evolution are far more likely to be derived from field magnitudes. The reason for this is partly one of sampling, for it would be difficult to determine the shape of the ancient lunar field from the few, thinly distributed sites sampled so far even if all the rocks collected had been oriented. Moreover, as continental drift has no relevance to the Moon, one of the most important uses for terrestrial palaeodirectional data has no lunar equivalent. The determination of palaeointensities, on the other hand, requires neither orientation nor dense geographical coverage. It may be true that a complete interpretation of magnitude data requires information on the corresponding field shape, but the lack of such information is not unduly restricting. For a dipole field, for example, field intensities vary by a maximum factor of only two (equator to poles).

The problem with this analysis, however, is that it assumes that the field intensities are actually determinable, whereas in practice this is only so for a relatively small proportion of samples. Moreover, as Hoffman and Banerjee (*Earth planet. Sci. Lett.*, **25**, 331; 1975) have now discovered, there is a whole class of lunar rocks (with no terrestrial analogue) having properties which "could easily render any palaeointensity determination of little value". When subjected to partial alternating frequency demagnetisation, certain crystalline rocks and breccias are found to have intensities of magnetisation which are non-reproducible at given a.f. fields; and in such cases the graph of natural remanent magnetisation against a.f. peak field has an unusual 'zig-zag' pattern. This behaviour has now been observed to varying degrees in many lunar rocks, and raises questions both as to its cause and as to the effects it is likely to have on the picture of the ancient field the rocks potentially provide.

As to cause, Hoffman and Banerjee have used both a.f. and thermal techniques to carry out a thorough study of lunar olivine basalt sample 15535—a rock in which the zig-zag behaviour is particularly marked. Perhaps the most surprising fact to emerge is that a substantial fraction of the total remanence in the basalt (that part responsible for the zig-zag pattern, in fact) is apparently confined to a single plane but is loosely pinned within that plane. When a.f. demagnetisation is carried out to a particular peak field, this 'zig-zag remanence' can take up any direction within the preferred plane; and when repeated demagnetisations are carried out at the same field, the zig-zag remanence will generally end up in a different direction each time. The remanence also varies in magnitude (hence the zig-zag), although neither peak a f. fields of 1,000 oersted nor moderate temperatures are sufficient to remove it completely.

The conclusion that Hoffman and Banerjee come to is that a high proportion of the basalt's total remanence is carried by a few large disk-shaped multi-domain grains which are aligned by the local magnetic fabric. Whether the same can be said for all other lunar rocks exhibiting zig-zag behaviour is not yet known. But if the loose pinning of remanence to a plane is a common feature, it follows that conventional methods of palaeointensity determinations are likely to be useless. The Thellier method, for example, depends on a comparison of natural and thermoremanent magnetisations which have had a similar history of demagnetisation. If a large component of the natural magnetisation is ultimately undemagnetisable or behaves at all randomly under demagnetisation, the technique collapses.

Fortunately, this phenomenon is unknown in terrestrial rocks, although palaeointensity measurements are often precluded by a multitude of other problems. Nevertheless, using his new anhysteretic remanent magnetisation technique, Shaw has managed to obtain some interesting ancient field intensity data from the R_3–N_3 polarity transition as recorded in the rocks of western Iceland (*Geophys. J.*, **40**, 345; 1975).

The results show that as the transition progressed from the fully reversed state to the fully normal state the field intensity generally decreased to very low values and then increased again. The lowest intensity recorded was equivalent to about 4% of the present value. But not all the intermediate dipole moments were low. When the geomagnetic pole reached the geographic equator it apparently remained there for a time while the dipole moment rose smoothly to a value about 21% higher than even the present fully 'normal' value, and then decreased equally smoothly to the very low values preceding the increase to the N_3 state.

Shaw's interpretation of this remarkable phenomenon is that the Earth's magnetic field could well have a third metastable state (intermediate, in addition to normal and reversed) in which the pole is temporarily fixed and the dipole moment is large. In the short periods (transitions) between metastable states, by contrast, the pole position changes rapidly while the dipole moment is small. Because the intermediate state is likely to be short, examples will be difficult to detect palaeomagnetically; but if confirmed as a general feature of the geomagnetic field, it will clearly place an additional constraint on theories of field origin. It will be a long time before such fine points can be made in connection with the lunar field.

Progress on penicillinase

from David R. Thatcher

A workshop was held at the University of Newcastle upon Tyne from April 16 to 18 to discuss the biochemistry of bacterial penicillinases.

PENICILLINASES, or as they are now known, β-lactamases, are enzymes which specifically deactivate penicillin by hydrolysis of the β-lactam ring. Antibiotic resistance in a number of pathogenic bacterial strains has been conclusively linked with the production of these enzymes. In recent years transmissible plasmids or R factors carrying β-lactamase genes have been responsible for the rapid spread of resistance among organisms previously sensitive to penicillin. Penicillinases are therefore of immense medical importance and a detailed knowledge of their structures and mechanisms will be crucial in the development of new approaches to penicillin chemotherapy.

The present uses of β-lactamases in pharmaceutical research were outlined by G. W. Ross (Glaxo, Greenford). He pointed out that as new prospective antibiotics are synthesised it is essential that they be exposed to as wide a spectrum of naturally occurring β-lactamases as possible. During the past few years the interest of the drug companies has shifted from the gram-positive producers like *Bacillus cereus*, to gram-negative producers like *Pseudomonas aeruginosa* and *Escherichia coli*, as the greater medical significance of the latter group and their R factors was realised. β-Lactamases from a large number of gram-negative sources have been characterised at Glaxo, using immunological techniques and a new isoelectric focusing method. Several of these enzymes were purified to homogeneity using an interesting single-step QAE-Sephadex column chromatography method. J. W. Dale (University of London) reviewed the range of penicillinase types determined by R factors, including a fascinating class of dimeric β-lactamases having a high specificity for oxacillin.

It is becoming increasingly apparent, however, that any fundamental advances in antibiotic design will be based on our physico-chemical understanding of the interaction of the drug with the target enzyme (in this case the cell wall transpeptidase) and any specific resistance mechanisms (in this case, penicillinase). Structural studies at the moment are still governed by the availability of large quantities of pure enzyme and consequently most of our information is confined to the β-lactamases of gram-positive super-producing strains of little medical importance. Complete amino acid sequence data are available on the enzymes from *Staphylococcus aureus* and *Bacillus licheniformis* with partial data on the sequence of the β-lactamase I of *Bacillus cereus* (80%) and preliminary data on the *E. coli* TEM enzyme. R. P. Ambler (University of Edinburgh) explained how the three gram-positive sequences known show a high degree of similarity to each other whereas the fragmentary data available on the *E. coli* TEM enzyme show little, if any, evidence for homology.

X-ray crystallographic analysis of two enzymes, the *E. coli* TEM enzyme (J. R. Knox, University of Connecticut) and the *S. aureus* penicillinase (D. W. Green, University of Edinburgh) has commenced and L. Sawyer and J. Moult (University of Edinburgh) presented a report of progress made up to now. It is hoped that low resolution structures will soon be completed for both enzymes.

The conformation of the *S. aureus* penicillinase in solution has been examined by difference spectroscopy, optical rotatory dispersion and circular dichroism (R. H. Pain, University of Newcastle upon Tyne). Gradually unfolding the protein in increasing concentrations of guanidine hydrochloride revealed a partially unfolded state(s) which although retaining all α-helical structure, was enzymologically inactive and appeared, on the basis of optical absorption and flow properties, to be expanded, with the 13 tyrosine residues fully exposed to solvent.

By analysing the equilibrium constants of the transitions between the native, partially unfolded and fully unfolded states, Pain and co-workers have been able to calculate the free energies of the two denatured states, with respect to the native state. These values are much lower than, for example, those found in the denaturation of ribonuclease or lysozyme. *S. aureus* penicillinase could in fact be described as a 'floppy' molecule and Pain has calculated that in solution up to 3% of the enzyme molecules are in the partially unfolded state(s). Conformational flexibility is becoming a characteristic property of β-lactamases or, more specifically, conformational changes provide the best explanation of a number of unusual pencillinase properties.

The search for residues involved in the catalytic site of β-lactamases continues without marked success. S. G. Waley (University of Oxford) presented $k_{\mathrm{CAT}}/K_{\mathrm{M}}$ against pH plots for the hydrolysis of benzylpenicillin by the β-lactamase of *B. cereus*. The usual bell-shaped plots appeared and these results were explained on the basis of the ionisation of groups in the catalytic centre (pK_{a}s of 5.5 and 8.6) affecting substrate binding. The group with a pK_{a} of 5.5 could either be a carboxyl or an imidazole side chain, Waley tentatively favouring the former on the basis of inactivation studies using Woodward's reagent. Waley also described a number of elegant inhibition experiments using penilloates (decarboxylation products of penicilloic acid) and showed that these compounds were much better inhibitors than the natural penicilloic acid products.

Stopped-flow spectrophotometric data (S. Halford, University of Bristol) using the *S. aureus* enzyme and a chromogenic substrate showed that in this case the bell-shaped plot of k_{CAT} against pH could arise by the change of one rate-determining step to another with pH. Rapid reaction kinetics resolved the reaction into three phases, the formation of (ES), conversion of (ES) to (EP) and the decomposition of (EP) to the free enzyme and product. Below pH 6.0 the reaction rate is limited by the conversion of (ES) to (EP) and above pH 6.0 by the decomposition of (EP).

Using a pH indicator in the assay system, Halford was able to show that proton release accompanied the (ES) to (EP) transition; a result which favours a general acid-base catalytic mechanism rather than a hypothesis involving the formation of a covalent acyl intermediate. If the release of this proton is involved in the rate-limiting decomposition of (ES), a primary isotope effect should be observed when the reaction is performed in D_2O. As no such effect was observed the rate of conversion of (ES) to (EP) probably also involves a rate-limiting conformational change after proton release.

R. Virden (University of Newcastle upon Tyne) and coworkers have been studying the effect of quinacillin inhibition on *S. aureus* penicillinase. This penicillin analogue is a substrate, albeit a poor one, yet it progressively inactivates the enzyme and on subsequent dilution into benzylpenicillin solutions the inhibited enzyme only slowly regains normal enzymic activity.

Kinetic analysis of this reversible inhibition has been performed and was explained in terms of quinacillin binding preferentially to an inactive conformation of the enzyme. Denaturation during quinacillin hydrolysis yields a covalent enzyme–substrate complex. Chemical analysis of this complex has not yet shown whether or not quinacillin is bound at a single site.

High resolution structural data on a β-lactamase will be invaluable in the interpretation of many of the kinetic and chemical modification data. Apart from the potential benefits to medicine, it looks as if future research on penicillinases will make many valuable contributions to our understanding of enzyme function in general.

articles

Lead and strontium isotopes in post-glacial basalts from Iceland

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We report here our Pb and Sr isotopic study of some post-glacial basalts (less than 12,000 yr old) from the southern part of Iceland, and use our new data to evaluate various proposed models for the genesis of volcanic rocks on Iceland. The interpretation for Icelandic rocks is also extended to the genesis of volcanic rocks on other oceanic islands of the world.

SAMPLES of basalts from Iceland (locations shown in Fig. 1) have been analysed at the NASA Johnson Space Center. Lead isotope compositions (Table 1) were analysed using the silica gel method, and the ^{206}Pb/^{208}Pb ratio of NBS 982 equal atom standard was routinely analysed as a monitor for the mass fractionation correction. During the study, our silica gel gave −0.12% mass fractionation per mass unit difference. CIT standard Pb determined by us gives ^{206}Pb/^{204}Pb = 16.637±0.010, ^{207}Pb/^{204}Pb = 15.482±0.010 and ^{208}Pb/^{204}Pb = 36.300±0.030 (2σ). These values agree well with 'absolute values' reported by Catanzaro[1]. The uncertainties (2σ) are believed to be better than ±0.1% for ^{206}Pb/^{204}Pb, ±0.15% for ^{207}Pb/^{204}Pb and ±0.2% for ^{208}Pb/^{204}Pb. Analysis of Sr compositions gives the ^{87}Sr/^{86}Sr ratio of NBS 987 standard as 0.71025±2 ($2\sigma_m$, based on 15 replicate analyses with a total range of 0.00012). The uncertainties quoted in Table 1 represent in-run precision. No replicate analysis was made.

Basic data

The isotopic compositions of Pb and Sr, and the concentrations of U, Th and Pb are presented in Table 1. Welke *et al.*[2] reported lead isotope data for some Icelandic igneous rocks of Miocene to recent age and found that the rocks could be divided into two groups based on the isotopic compositions. The less radiogenic group (Group I) includes older (7–16 Myr) rocks from eastern, northern and north-western Iceland and post-glacial rocks from the Northern Neovolcanic Zone (Fig. 1); the more radiogenic group (Group II) consists of younger (< 3 Myr to recent) rocks from western, south-western and central Iceland, including Surtsey. We have recalculated their ^{206}Pb/^{204}Pb data to 'absolute' ratios based on their Broken Hill galena lead standard. The new values are plotted along the ^{206}Pb/^{204}Pb axis of Fig. 2. The reason for doing so is that their ^{207}Pb/^{204}Pb values are not accurate enough to be useful and the present technique for acquiring high quality data enables us to measure more accurate ^{207}Pb/^{204}Pb ratios. Consequently, we can determine more accurately the Δ^{207}Pb/Δ^{206}Pb slope which is vital in data interpretation.

The regression line in Fig. 2 was calculated using the York method[3]. For replicate analyses, such as I-48 and I-103, the the average values were used. The slope of this regression line is 0.09±0.02 (2σ) which is similar to that for other oceanic islands[4]. For example, the slope (Δ^{207}Pb/Δ^{206}Pb) for the Canary Islands samples is 0.110±0.014 (2σ), with a total range of ^{206}Pb/^{204}Pb variation about 5%.

The ^{87}Sr/^{86}Sr ratios of the rocks (I-92, I-17, I-103 and I-115) from the Reykjanes Peninsula (Central Neovolcanic Zone) have a narrow range of 0.70305 to 0.70315. Hart *et al.*[5] also reported Sr isotopic compositions for four rocks from the same general area and they range from 0.70301 to 0.70308 (IC-47, IC-62, IC-58 and R-11), after making an adjustment of NBS 987 Sr standard value.

A sample from the Snaefellsnes Zone (SN-1) has a ^{87}Sr/^{86}Sr ratio of 0.70345±5, substantially greater than values from the Central Neovolcanic Zone. Hart *et al.*[5] and O'Nions and Pankhurst[6] also reported larger values (about 0.70330) for the Snaefellsnes rocks.

The samples from Eldgja (I-48) and Hekla (I-136A) of the Eastern Volcanic Zone (EVZ) also have larger ^{87}Sr/^{86}Sr ratios than the volcanic rocks from the Central Neovolcanic Zone.

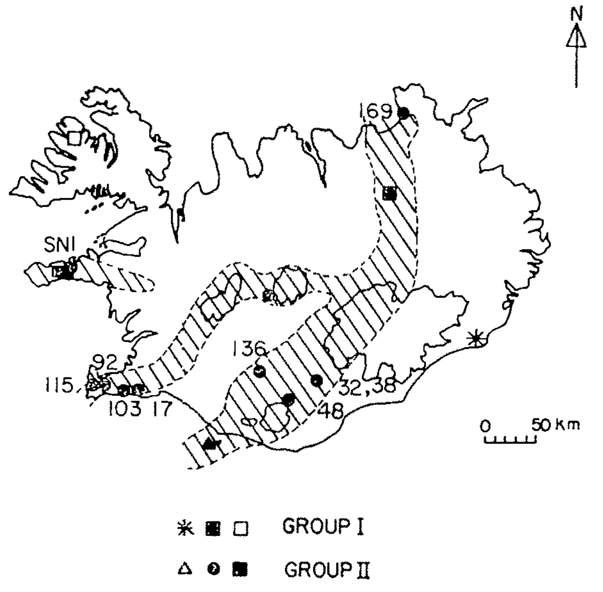

Fig. 1 Sampling localities. The localities of samples analysed by Welke *et al.*[2] are also shown.

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Table I Lead and strontium isotopic compositions and U, Th and Pb concentrations in Iceland basalts

Sample no.	Location	Concentration (p.p.m.) U	Th	Pb	$^{232}Th/^{238}U$	$^{238}U/^{204}Pb$	Atomic ratio $^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$	$^{87}Sr/^{86}Sr$*
I-48	Eldgja lava, Eldgja	0.553	1.84	1.58	3.37	26.1	19.260 19.249	15.558 15.551	38.925 38.908	0.70336±4
SN-1	Berserkjahroam flow, Snaefellsnes	0.319	1.03	1.04	3.27	22.8	19.110 19.128	15.521 15.536	38.697 38.725	0.70345±5
I-136A	1913 Lamfitjarhraum eruption north of Hekla	0.584	1.82	1.61	3.14	26.8	19.008 19.012	15.510 15.506	38.516 38.506	0.70323±3
I-92	Arnarsetur, Reykjanes Peninsula	0.148	0.433	0.677	2.97	16.2	18.761 18.759	15.487 15.486	38.307 38.304	0.70314±3
I-17	Krysuvikurhraum, Reykjanes Peninsula	—	0.445	0.576	—	—	18.751	15.503	38.337	0.70315±5
I-103	Near Isolfsskali, Reykjanes Peninsula	0.0844	—	0.414	—	14.9	18.680 18.661	15.518 15.502	38.336 38.278	0.70305±6
I-115	Near Langholl, Reykjanes Peninsula	0.0644	—	0.475	—	9.99	18.640	15.501	38.294	0.70309±6
I-32	Laki	0.304	0.985	1.01	3.28	21.9	18.474 18.472	15.476 14.471	38.204 38.192	0.70314±5
I-38	Laki	0.594	1.87	1.30	3.19	33.4	18.478	15.476	38.222	0.70322±4
I-169	Leirhafnarfjall						18.466	15.485	38.178	

*NBS-SRM987 value = 0.71025±2 ($2\sigma_m$)
All errors quoted represent in-run precision $2\sigma_m$.

O'Nions and Gronvold[7] also reported bigger ratios for icelandites from both Torfajökull and Hekla areas.

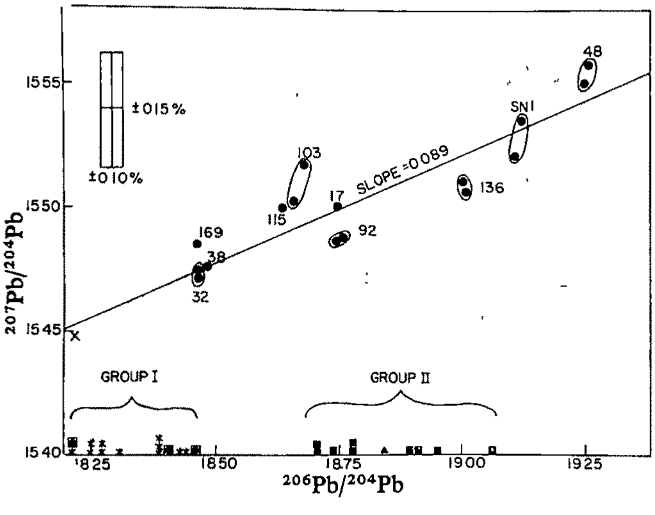
Two samples from the Laki area (I-32, I-38) have similar Pb but perhaps slightly different Sr isotopic compositions. Their U and Th concentrations are, however, different by a factor of two.

Our Sr data are in close agreement with those of Hart *et al.*[5] in all comparable cases. Hart *et al.*[5] and O'Nions and Pankhurst[6] pointed out that the values reported by O'Nions, Pankhurst and others[6-9] are systematically greater by about 0.00010 than those of Hart *et al.* after the adjustment of Sr standard values was made.

The data in Table 1 are arranged in a general trend of decreasing radiogenic nature of Pb isotopes. Most of the Sr isotope ratios (except I-38) follow the same trend. But, it seems that the radiogenic nature of Sr isotopes also parallels the concentrations of U and Th in the rocks, for example, I-48, SN-1, I-136A and I-38 have larger $^{87}Sr/^{86}Sr$ ratios, as well as U and Th contents, than others.

A very uniform value of $^{87}Sr/^{86}Sr$ was emphasised by O'Nions and Gronvold[7] and Hart *et al.*[5] for most of the post-glacial basalts from Iceland. In contrast, our present data show that the basalts with alkali affinity from the flanking zones have a more radiogenic nature.

Fig. 2 A $^{207}Pb/^{204}Pb$–$^{206}Pb/^{204}Pb$ plot of our new data. $^{206}Pb/^{204}Pb$ values for samples of Welke *et al.*[2] were corrected to absolute values and plotted along the $^{206}Pb/^{204}Pb$ axis for comparison. Symbols used are the same as in Fig. 1. A basalt from the Reykjanes Ridge[6] (×) is also plotted for reference.



O'Nions and Pankhurst[8] observed a secular variation in the Sr isotope composition of Icelandic volcanic rocks. Older rocks are generally more radiogenic than the younger rocks. This contradicts the trend found by Welke *et al.*[2] from their Pb isotope study.

Existing models

The existing model interpretations for Icelandic Pb and Sr isotopic data may be grouped into two categories: disequilibrium (Rayleigh) partial melting of a homogeneous mantle source or crustal contamination of a partial melt from a homogeneous mantle source; and equilibrium partial melting in various domains of heterogeneous mantle source, with mixing of the resulted melts permissible.

The Pb isotopic compositions of Group I rocks (older) were regarded by Welke *et al.*[2] as representing the isotopic character of the mantle source. They thought that the younger magmas of Group II might have picked up radiogenic leads by highly selective wall rock reactions on their way to the surface. The contaminants (wall rocks) were believed to be slightly older yet highly differentiated rocks, such as granophyre or obsidian underlying the post-glacial volcanic piles. To explain the large difference in $^{206}Pb/^{204}Pb$ ratios but very narrow range of $^{207}Pb/^{204}Pb$ ratios between the two groups, they suggest that the acquired radiogenic lead must be relatively young (~20–30 Myr). Furthermore, the possibility of contamination with ancient lead, as commonly observed in continental areas, is totally ruled out.

O'Nions and Pankhurst[8] observed secular variation of the initial $^{87}Sr/^{86}Sr$ ratios among the Icelandic volcanic rocks. The ratios decrease progressively from 0.7036 at 15 Myr BP to 0.7033 at 2 Myr BP (E and A standard = 0.70815±1). They proposed three possible ways of interpretation:

(1) Disequilibrium (Rayleigh) partial melting of a homogeneous mantle source. During partial melting process, there is no isotopic equilibration between the liquid and the residual solid phases. Amphibole and phlogopile which have more radiogenic Sr and higher Rb/Sr ratios preferentially enter the early melt. The secular variation may be caused by Rayleigh melting of a single source through time (considered unlikely[8] regarding to the upwelling and spreading rates of the mantle materials); or by increase in degree of partial melting of continuously replenished mantle (thus constant mantle composition) over the past 15 Myr.

(2) Partial melting of heterogeneous mantle sources.

(3) Mixing of two or more mantle sources and progressive change of the mixing ratio with time. This interpretation contains two alternative possibilities: Contamination (or mixing)

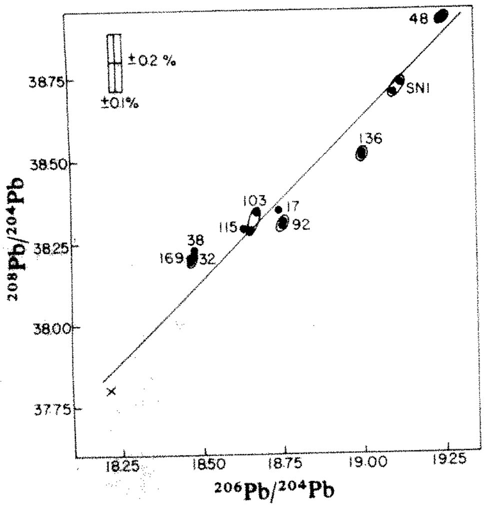


Fig. 3 A ^{208}Pb/^{204}Pb–^{206}Pb/^{204}Pb plot of our new data. Symbols used are as in Fig. 1. A basalt from the Reykjanes Ridge (×) is also plotted for reference.

of upwelling normal mid-ocean ridge basalt magmas through a segment of mantle enriched in incompatible elements and radiogenic ^{87}Sr, with the contamination effect decreasing with time; or mixing of an upwelling mantle plume (relatively enriched in incompatible elements and radiogenic ^{87}Sr) with low velocity zone material, with the plume activity decreasing with time since 15 Myr BP.

Implications of new data

The linear array of our Pb isotopic data, which yields a slope of 0.09±0.02 (2σ), argues against the interpretation of Welke *et al.* that younger magmas were contaminated by older Icelandic volcanics, because the regression line produced by such contamination would probably have a shallow slope[2] of 0.046 due to recent radiogenic lead growth from ^{235}U and ^{238}U. Moreover, our data suggest that if Rayleigh melting in a homogeneous mantle or equilibrium partial melting in a heterogeneous mantle was responsible for the observed Pb isotopic variation among the Icelandic samples, the age for the source rock is about 1,500±500 (2σ) Myr.

Wall rock reaction and Rayleigh melting were considered to enrich the radiogenic daughters in the melts, thus the general increase of ^{206}Pb/^{204}Pb ratios accompanied by the decrease of ^{87}Sr/^{86}Sr ratios in the younger magmas[8] indicates that the sub-Icelandic mantle is not a simple system. Our data cannot be satisfactorily explained in terms of a homogeneous mantle source. Furthermore, the ^{87}Sr/^{86}Sr values for alkali basalts from Surtsey and Heimaey are not higher than those of olivine tholeiites from the Central Neovolcanic Zone[9]. This is also not consistent with what we expect from the Rayleigh melting; the problem is discussed in detail elsewhere (ref. 10 and S.-S.S., M. Tatsumoto and J.-G. Schilling, to be published). This leaves models which involve heterogeneous mantle sources for the Icelandic magmas.

A mixing model in which upwelling mid-ocean ridge basalt magmas mix with the overlying mantle enriched in incompatible elements is difficult to reconcile with our new data. Suppose that part of mantle is characterised by large ^{87}Sr/^{86}Sr and small ^{206}Pb/^{204}Pb as suggested by the older Icelandic volcanics. The decrease of involvement of this mantle domain in the magma generation with time should reduce both observed ^{87}Sr/^{86}Sr and ^{206}Pb/^{204}Pb ratios, because the ^{206}Pb/^{204}Pb ratios of normal ridge basalts are low (ref. 4 and unpublished work of S.-S. S. and others) (~18.20 for basalts from the Reykjanes Ridge.)

In contrast, most younger Icelandic rocks have greater ^{206}Pb/^{204}Pb ratios than the older rocks. To avoid this problem, the overlying mantle must be heterogeneous in Pb isotopic composition. It requires a very radiogenic character for the southern part of Iceland, except for the Laki samples which seem to be similar to the mantle that produced older Icelandic magmas.

Our choice of model is strongly influenced by a Pb isotope study of the Reykjanes ridge basalts (unpublished work by S.-S.S. and others). In this model, mixing of the Icelandic mantle and the normal mid-ocean ridge mantle (the low velocity zone?) along the Reykjanes Ridge is strongly suggested. This is somewhat similar to the mantle plume–low velocity zone mixing model proposed by Schilling[11]. We consider that the Icelandic magmas were probably not derived from a pure mantle plume component. Instead, they were formed by a mixing of the plume source and the material of low velocity zone as suggested by O'Nions and Pankhurst[8]. The involvement of the plume component is believed to have decreased with time since 15 Myr BP. Good evidence to support this mixing model is that all Pb isotope data from Iceland and the Reykjanes Ridge form a linear array in both ^{207}Pb/^{204}Pb–^{206}Pb/^{204}Pb and ^{208}Pb/^{204}Pb plots.

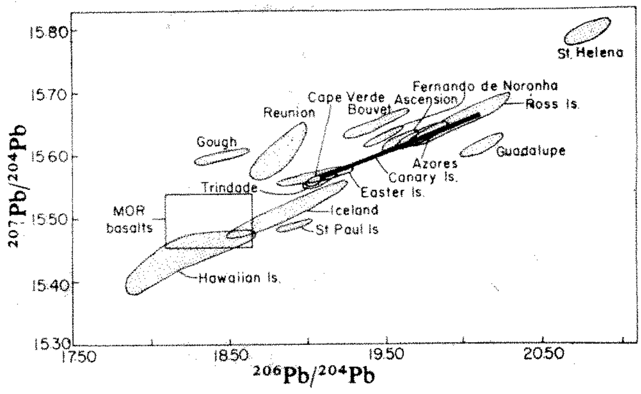
Implications of our model

If the decrease of ^{87}Sr/^{86}Sr ratios in younger magmas[8] is used as an indication of decreasing plume activity, then both our data and those of Welke *et al.* suggest that the plume component itself is not homogeneous in Pb isotopic composition. Our Sr data also indicate that the post-glacial magmas do not have uniform ^{87}Sr/^{86}Sr ratios. In general the recent 'plume' material that upwells in the southern part of Iceland seems to be more radiogenic in Pb isotopic composition than that in northern Iceland. In the present interpretation, the basalts from Snaefellsnes, Eldgja and Hekla are explained as being derived from less mixed (less diluted) or unmixed plume sources.

Being limited by the inherent analytical uncertainty in the Pb isotope analysis and the availability of the Pb data for Icelandic rocks, it is difficult, if not impossible, to characterise the Pb (or Sr) isotopic composition for the plume component at this time. Consequently, the evolutionary history of the plume source cannot be well defined in terms of any model such as the two-stage model (see, for example, refs 4 and 12).

The Icelandic Pb isotopic study has a profound impact on the interpretation of Pb isotopic data of the volcanic rocks in oceanic islands. As shown in Fig. 4, most oceanic island leads have regression lines pointing toward the field of mid-ocean ridge basalts[4,7]. These data were previously interpreted in terms of a modified two-stage model and a mantle-mixing model[4]. Since most of the oceanic islands including Iceland (Fig. 4) have been suggested as the locations of plume uprising[13], it is possible that some of the regression lines of the oceanic islands also represent mixing lines similar to the case of Iceland. Consequently, they do not have an age significance as in a two-stage model. But our present state of knowledge does not rule out the possibility of a two-stage model.

Fig. 4 A general picture of the Pb isotopic compositions for the oceanic islands. Data from Sun[4] and unpublished data.



We thank Dr N. Hubbard of NASA-JSC for supplying samples and discussions. S.-S.S. thanks Dr M. Tatsumoto for allowing him to join in the study. The support of a fellowship at the Lunar Science Institute (S.-S.S.) and an NRC Research Associateship at the Johnson Space Center (B.J.) are acknowledged. The Lunar Science Institute is operated by the Universities Space Research Association under contract with NASA.

Received January 6; accepted April 28, 1975.

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Kinky helix

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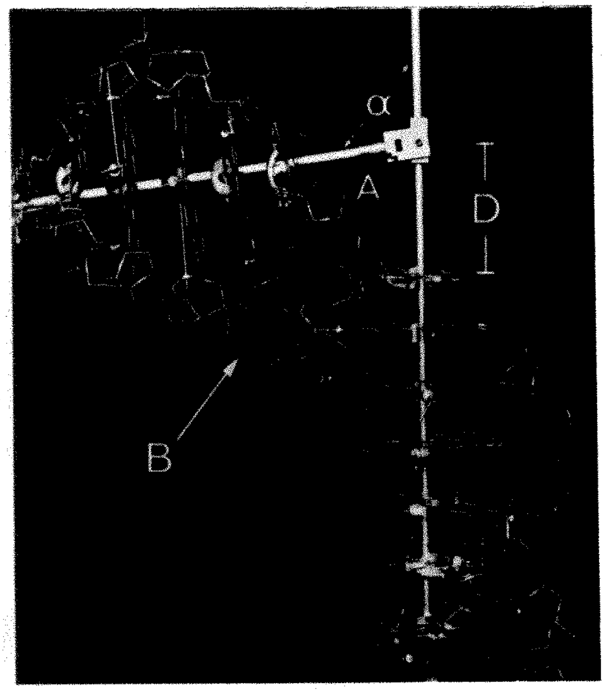
DNA in chromatin is highly folded. Is it kinked? And does it kink in other situations?

CHROMATIN is the name given to chromosomal material extracted from the nuclei of cells of higher organisms. It consists mainly of DNA and a set of small rather basic proteins called histones. Other proteins and RNA are present in lesser amounts (see for example ref. 1). Early X-ray work (for review see ref. 2) suggested that there was a structure in chromatin which repeated at intervals of about 100 Å. More recent work using nucleases[3,4] has shown that the DNA in chromatin exists in some regular fold which repeats every 200 base pairs, the best value currently being 205±15 base pairs[5].

The most cogent model for chromatin has been put forward by Kornberg[6] who suggested that the basic structure consists of a string of beads each containing two each of the four major histones, each bead being associated with about 200 base pairs of DNA. Linear arrangements of beads (in a partly extended form) were first seen in the electron microscope by Olins and Olins[7] and called by them ν-bodies. The exact diameter of a bead in the wet state is rather uncertain but it is probably in the region of 100 Å. Kornberg's model suggested that DNA, when associated with histone, is folded to about one-seventh of its length. This is the value deduced by Griffith[8] from electron micrographs of the mini-chromosome of the virus SV40. A similar value has been obtained by Oudet *et al.*[9] from measurements on adenovirus 2. Other compact models have been proposed by van Holde *et al.*[10] and Baldwin *et al.*[11].

Fig. 1 General view of a model of a kink, taken from the side. For this model $d = 0$, $\alpha = 98°$, $D = 8$ Å and $\theta = 23°$ (see text). The two short lengths of backbone, connecting the two stretches of straight helix, can be seen at A. The region of van der Waals' contacts between backbones, which limit the kink angle α, is near B.



Thus the DNA in chromatin, even at this first level of structure, must be folded considerably since its length is contracted to about one-seventh. Moreover, the basic repeat of 200 base pairs (which is 680 Å long in the B form of DNA) must be folded into a fairly limited space having the dimensions of about 100 Å^3 (ref. 6).

We have found it very difficult to estimate just how much energy is required to bend DNA "smoothly" to a small radius of curvature, say 30–50 Å, bearing in mind that these numbers are not many times greater than the diameter of the DNA double helix, which is about 20 Å, and that bending a helix destroys its symmetry. We have formed the impression that the energy might be rather high. We therefore asked ourselves whether the folded DNA may consist of relatively straight stretches joined by large kinks. This paper describes a certain type of kink which can be built rather nicely and has interesting properties.

The stereochemistry of a kink

No doubt other types of kink could be built, but we have concentrated on one special type which we consider to be rather plausible. We have assumed that all the base pairs of the double helix are left intact (so that no energy is lost by unpairing them), that the straight parts of the DNA on each side of the kink remain in the normal B form, but that at the kink one base pair is completely unstacked from the adjacent one. Thus at each kink the energy of stacking of one base pair on another is lost. Naturally all bond distances and angles (including dihedral angles) have to be stereochemically acceptable.

We find that, given these assumptions, one can convincingly build a neat kink, having a large angle of kink, in one way only; or, more strictly, in a family of ways all very similar to each other. The double helix is bent towards the side of the minor groove. This can be seen in the photograph of one such model shown in Fig. 1.

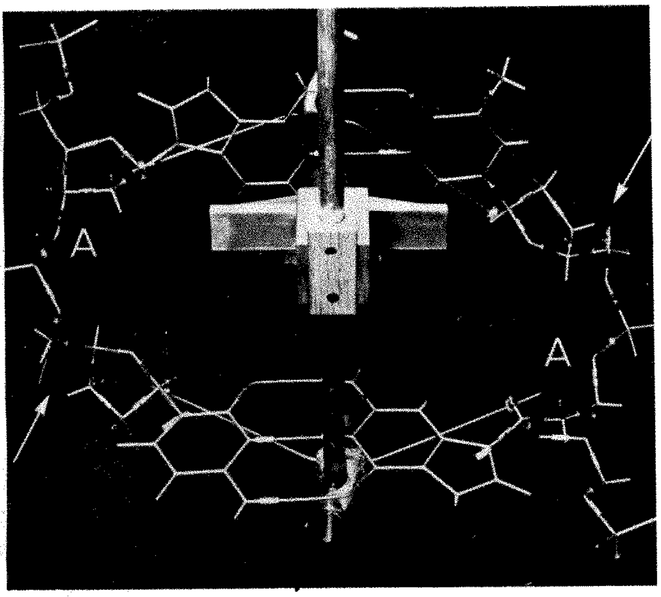


Fig. 2 View of part of the model of Fig. 1 taken approximately looking down the pseudo-dyad. It shows two base pairs, one on either side of the kink. The rest of the model has been blanked out for easier viewing. The two arrows point to the C_4'–C_5' bonds at which the chain conformation is changed by kinking—see Fig. 3. The letters A correspond to the region marked A in Fig. 1.

The structure can be built with an approximation to a dyad axis passing through the kink, though we cannot see any strong reason why such symmetry is essential in chromatin. A partial view looking along the pseudo-dyad is shown in Fig. 2.

To our surprise the configuration of the backbone at the kink can be made similar to that of the normal backbone of the B form except that the conformation at the C_4'–C_5' bond in the sugar is rotated 120° about this bond, going from one of the possible staggered configurations to another one as shown in Fig. 3. (We have arbitrarily kept the same pucker of the sugar ring as is found in the B form of DNA.)

The structure of the backbone at the kink is not one of the preferred configurations that have been listed[12,13] since these involve a weak CH · · · O close contact between the hydrogen attached to either C_6 of a pyrimidine or C_8 of a purine and the O_5' of the sugar, and by its very nature the type of kink we have assumed cannot have this at every position. In our model this contact is absent for one base of each of the base pairs immediately adjacent to the kink. As explained above, however, the backbone configuration is sufficiently close to that of the B form of DNA (except for the torsion angle about C_4'–C_5') that we feel it is acceptable stereochemically.

The nature of the base pairs on either side of the kink is immaterial to the model though presumably the energy required to unstack these base pairs will depend to some extent on their composition. We have found it difficult to estimate the free energy involved. It is probably a few kilocalories. It is obviously desirable that this figure should be determined as accurately as possible since the ease of making kinks depends on just how big it is. Another important question is how much a DNA double helix can be bent before it kinks. If we denote the mean curvature by κ (where κ equals the reciprocal of the radius of curvature) then we would expect the energy of deformation of a uniformly bent helix, per unit length, to increase at least as fast as κ^2. For a kinked helix, on the other hand, this energy increases only as κ. In this case κ is the mean 'curvature' of the segmented double helix which is proportional to the number of kinks per unit length. Thus, as is intuitively obvious, at small κ the double helix will bend, while at large κ it will kink. The value we should like to know is the radius of curvature at which it changes from bending to kinking.

There is probably an appreciable activation energy to the process of making a kink since the C_4'–C_5' bond must pass through the eclipsed configuration. For this reason we consider kinks of this type with a kink angle of about half the full 100° to be unlikely.

Common features of the family

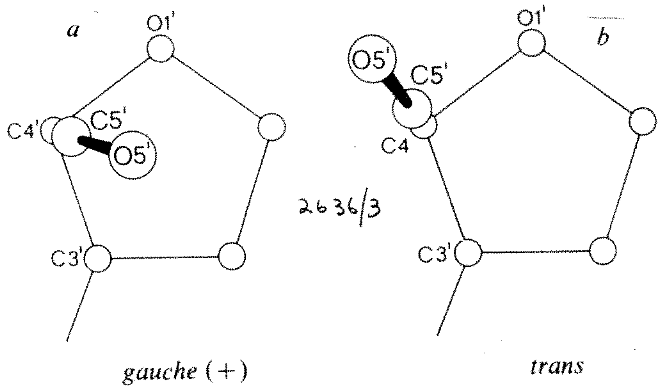
A number of very similar structures can be built along these lines and it is not obvious which is to be preferred. They all have certain features in common.

(1) The axes of the two straight parts of the DNA do not necessarily intersect exactly, but may be separated by only a small distance, d, typically about 1 Å or less. Note that d has a sign. (2) The angle between the two axes, α (projected, if necessary, on to a plane perpendicular to the line joining their points of nearest approach) is easily made more than 90° but approaches 100° with difficulty. The model shown in Fig. 1 has $\alpha=98°$. At the maximum angle (for any particular model) the backbone of one straight part starts to touch the backbone of the other chain of the other straight part. This contact, marked B in Fig. 1 has a (local) dyad axis. (3) If we define the kink point as the point where the local helix axes, on either side of the kink, intersect (or, if they do not intersect, then the midpoint of the shortest straight line between the axes) then the distance, D, from the kink point to the plane of the nearest base pair is appreciable and typically in the region of 7–8 Å.

To introduce our fourth point we must first consider the relationship between three successive straight portions; that is, two kinks in succession. We assume that every kink is exactly the same. The structure formed will depend on the precise number of base pairs between the kinks. (It should be remembered that the B form of DNA has an exact repeat after ten pairs.) For example, if there are ten (or a multiple of ten) base pairs between two kinks, the structure will bend round into, very roughly, three sides of a square. If there are five base pairs between kinks (or an odd multiple of five) then the structure will approximate to a zigzag. In short the dihedral angle for three successive straight portions will depend on the exact number of base pairs between the two adjacent kinks.

We can now state our fourth point. (4) The kink imparts a small negative twist to the DNA. This is most easily grasped by imagining that the kink is made in two steps; first, the two base pairs to be unstacked are unstacked in the axial direction without kinking the backbone—this

Fig. 3 Diagrams of the deoxyribose ring showing the approximate conformation at the C_4'–C_5' bond (a) in the normal straight B form of DNA, and (b) in the proposed kink; the two sugars affected are marked in Fig. 2.



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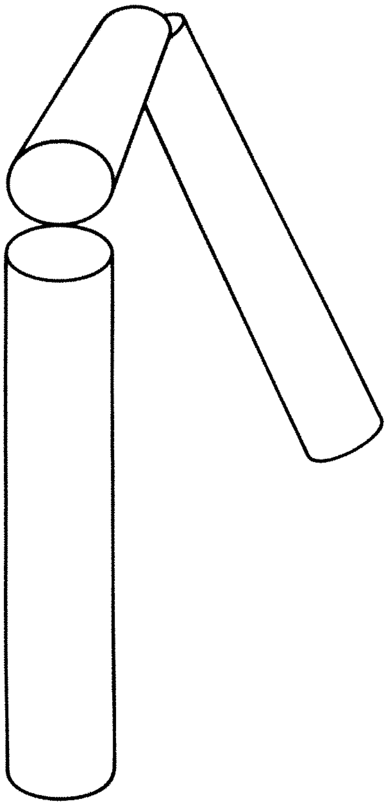

Fig. 4 Each cylinder represents a length of straight double helix. When there are ten base pairs (or an integer multiple of ten base pairs) in the middle stretch the kink has a left-handed configuration, as shown. The dihedral angle used in this paper is zero for the *cis* conformation. Its sign agrees with the usual convention that positive values less than 180° correspond to a right-handed configuration.

reduces the twist of 36° between these residues to about 10–20°—and second, that this extended structure is then kinked. The result is that if successive kinks are made at intervals of $10n$ base pairs (where n is an integer) the DNA instead of folding back to form a 'circle' follows instead a left-handed helix, though naturally a kinked helix made of straight segments (see Fig. 4).

The exact dihedral angle associated with three successive straight stretches depends somewhat on the precise details of the kink but is typically about $(m.36° - \theta)$ where m is the number of base pairs between the two kinks and θ, the dihedral correction angle, is not far from 15–20°. A very small rotational deformation of the straight portions could, however, alter this figure a little so the exact value in chromatin (if it does indeed consist of kinked helices) will probably be imposed by the histones.

(5) For any model there is a smallest number of base pairs between two adjacent kinks. For models of this family this number is usually three. In particular, the model illustrated in Fig. 1, for which $\alpha = 98°$, can be built with three base pairs between two kinks but not with only two base pairs there. This is probably true for all models of this type for which α is greater than 90°. A model with three base pairs between two kinks exposes these base pairs rather effectively.

It is easy to see that six parameters are needed to describe the relationship between any two (equal) stretches of straight double helix. If these stretches are related by a dyad axis through the kink point then only four parameters are required. These can conveniently be taken to be the four used above: d, α, D and θ.

Another family of models can be made with the kink on the side of the major groove, but such structures have a smaller angle of kink and seem rather awkward to build. We have not explored them further. A rather different type of kink, in which a base pair is undone, has been suggested by Gourévitch *et al.*[14].

The occurrence of kinks

The idea that the fold of DNA in chromatin was based on a unit of 10 base pairs was originally suggested to us by experimental evidence discovered by our colleague, Dr Markus Noll. Noll[15] has shown that the digestion of native chromatin with the nuclease DNase I produces nicks in the DNA which tend to be spaced multiples of 10 bases apart. This suggests that the DNA is folded in a highly regular way and is probably mainly on the outside of the structure[6,15]. More recent work by Noll and Kornberg (unpublished) using micrococcal nuclease points to a structural repeat at intervals of 20 base pairs. Thus a rather neat model for the most compact (wet) form of chromatin can be made in which the DNA is kinked through about 95–100° every 20 base pairs, giving a shallow kinked helix having 10 straight stretches of DNA in each 100 Å repeat. The middle stretches of this repeat would be largely protected by the histones of the bead, the flanking stretches less so. Whether this very simple model is basically correct remains to be seen.

Obviously we should ask whether DNA is kinked in other situations. One interesting possibility is that when the *lac* repressor binds to the operator site on the DNA the double helix becomes kinked. It has been shown by Wang, Barkley and Bourgeois[16] that this binding unwinds the helix by a small angle, either about 40°, or, more likely, about 90° (the value depending on the amount of unwinding assumed to be produced by the standard agent, ethidium bromide). As they point out, this is too small to allow the formation of a Gierer-type loop[17]. It is, however, just what one would expect from a small number of kinks since each kink of the kind we have described unwinds the double helix by about 15° to 25°. For example, an attractive zig-zag model can be imagined with four kinks, each spaced about five base pairs apart. This model places the two sequences related by a dyad, each of six consecutive base pairs, on either side of the first and last kinks (see Fig. 5). In this position, being near a kink, they are more exposed than they would be in a stretch of unkinked DNA.

In essence, kinking may be a way of partly exposing a small group of base pairs without too great an expenditure of energy. The exposed side of each of these base pairs is that normally in the major groove. The kink has the effect of displacing one of the phosphate–sugar backbones which normally make up the two sides of this groove. The specific pattern of hydrogen bonding sites in the major groove is thus made more accessible for a few base pairs on either side of a kink. A kink may therefore turn out to be a preferred configuration of DNA when it is interacting specifically with a protein.

Kinks may be suspected in all cases where double-stranded DNA has been shown to adopt a more compact

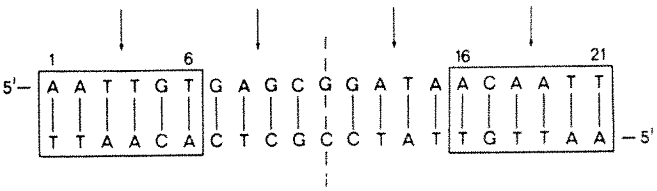
1
6
16
21
5'– A A T T G T G A G C G G A T A A C A A T T
T T A A C A C T C G C C T A T T G T T A A –5'

Fig. 5 The minimal base sequence of the *lac* operon, taken from Gilbert and Maxam[18]. The dotted line marks the pseudodyad in the base sequence. The two sets of consecutive base pairs, related by the dyad, are boxed, The arrows show one choice of positions where kinks might occur.

state than the normal double helix. Obvious examples are the folded chromosomes of *Escherichia coli*[19,20] (and no doubt other prokaryotes), the folded DNA in viruses, the ψ phase of naked DNA discovered by Lerman[21] and the shortened form of DNA in alcoholic solutions as described by Lang[22].

One should also ask whether kinks occur spontaneously, as a result of thermal motion, in double-stranded DNA in solution. The frequency at which this occurs clearly depends on the free energy difference involved. If this were, say, about 4 kcalorie then there should be one kink in about 800 base pairs which could be appreciable. Such kinks would occur mainly between A–T pairs. If the free energy were as high as 6 kcalorie this would produce one kink in about every 22,000 base pairs, which would be more difficult to detect.

At the present we have no compelling evidence which shows that DNA in chromatin is kinked rather than bent nor that kinks exist in DNA in other contexts. Nevertheless our model seems to us sufficiently attractive to be worth presenting now for consideration by other workers in the field. Kinks, if they occur, have at least two possible advantages. It has always been a puzzle how to construct hierarchies of helices in a neat way, since bending an existing helix necessarily distorts its regular structure. This distortion becomes more acute as the basic helix is coiled at higher and higher levels. A kink allows such deformations to be local rather than diffuse and makes it easier to build hierarchical models which are neat stereochemically. The other advantage is that, at a kink, several base pairs may be more easily available for specific interaction with a protein. If kinks in DNA exist they will surely prove to be important.

We thank our colleagues Drs R. D. Kornberg, M. Noll and J. O. Thomas for communicating their results to us before publication. We also thank them and our other colleagues for many useful discussions on chromatin structure.

Received April 25; accepted May 6, 1975.

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Isolation of histone genes from unfractionated sea urchin DNA by subculture cloning in *E. coli*

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Histone genes have been cloned selectively in Escherichia coli *directly from unfractionated sea urchin DNA using labelled mRNA probe. The cloning method used is potentially applicable for isolating any genetic sequence for which a probe is available. The several eukaryotic gene fragments introduced into bacteria by this method collectively contain all or nearly all of the DNA sequences represented in histone mRNA.*

THE ability to isolate eukaryotic DNA regions that code for specific gene products is vital to the understanding of gene organisation and function in higher organisms. Previously, separation of such regions from the bulk of eukaryotic DNA has been possible only when their nucleotide sequences have been highly repetitive or have had distinguishing physical properties, such as a skewed base-pair ratio[1–6]. But now DNA molecules from prokaryotic and eukaryotic sources can be cloned in *Escherichia coli* as part of a bacterial plasmid replicon[7–13] or bacteriophage genome[14]. So far the eukaryotic genes introduced into bacteria have been either purified species of DNA separated from the bulk of the chromosome (such as *Xenopus laevis* rDNA)[8,9], or non-fractionated, by randomly inserted fragments of eukaryotic DNA (such as *Drosophila melanogaster* DNA[12,14]). Such studies have shown that *X. laevis* rDNA is transcribed in *E. coli*[8].

Since the histone-coding DNA sequences of sea urchins code for an identifiable set of mRNAs and proteins[15,16], they are of particular value for studies of macromolecular synthesis by eukaryotic DNA cloned in *E. coli*. Similarly, cloning of DNA segments carrying histone genes would provide an important tool for investigating the organisation of the reiterated histone DNA sequences on the eukaryotic chromosome[17,18]. Histone genes have already been separated from bulk sea urchin DNA by physical methods[6]. We report here the isolation of histone genes directly from unfractionated sea urchin DNA by subculture cloning in *E. coli*. We used a radioactively labelled histone mRNA probe to identify bacterial clones containing histone genes. This procedure is applicable to the selection of any prokaryotic or eukaryotic DNA sequence for which a specific RNA probe is available.

Selection of histone DNA plasmids

A mixture of either unfractionated *Lytechinus pictus* or *Strongylocentrotus purpuratus* sea urchin DNA with pSC101 plasmid DNA (ratio 5:1) was cleaved by the *Eco*RI restriction endonuclease and ligated as described previously for *X. laevis*

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Table 1 Purification of sea urchin histone DNA-pSC101 chimaeras by subculture cloning

Sequential subculture steps	Detergent lysis hybridisation: c.p.m. hybridised per µg DNA / c.p.m. hybridised per µg *L. pictus* DNA	Purification ratio	CCC–DNA hybridisation: c.p.m. hybridised per µg DNA / c.p.m. hybridised per µg *L. pictus* DNA	Purification ratio
(1) *L. pictus*-pSC101 transformants	5.3/70.6	0.075	2.3/20.8	0.110
(2) Last positive row	58/67.4	0.87	7.0/20.8	0.337
(3) Grid row 1	86.6/54	1.60	13.7/20.8	0.659
(4) Grid column 4	52.2/54	0.97	10.4/20.8	0.5
(5) Grid intercept well	181/23.5	7.70	153/20.8	7.33
(6) Pool of intercept colonies	91/89.6	1.11	62/20.8	2.98
(7) Row A of pool	104/18.6	5.61	155/20.8	7.46
(8) Colony no. 8 (pLpl)	—	—	857/20.8	41.8

The presence of histone–DNA sequences among *E. coli* transformants was monitored by hybridisation of sea urchin histone ^{3}H-mRNA to plasmid chimaera DNA. For hybridisation, 50-ml overnight cultures of bacteria were subjected to detergent lysis[21], the chromosomal DNA was pelleted by centrifugation at 20,000*g* for 30 min, and the supernatant was treated with Proteinase K (EM Laboratories, Elmsford, New York) (50 µg ml^{-1}) at 37 °C for 30 min. The lysate was extracted twice with equal volumes of phenol and once with chloroform. Cold ethanol (2.5 volumes) was added to the aqueous phase and a precipitate was allowed to form at −20 °C for at least 2 h. The voluminous precipitate was collected by centrifugation and resuspended in 1.0 ml of water. NaOH was added (final concentration 0.5 N) and the solution was allowed to stand at room temperature for at least 30 min to accomplish denaturation of plasmid DNA. The solution was neutralised and loaded on to nitrocellulose filters[17,22]. Filters loaded with aliquots of DNA were hybridised with either sea urchin ^{3}H-histone mRNA at 52 °C or at 37 °C with complementary RNA (cRNA) transcribed[34] from pSC101 DNA by *E. coli* RNA polymerase (the enzyme was a gift of Dr K. Squires); both hybridisation reactions were carried out in 6×SSC containing 50% formamide for equal times ranging from 30 min to 18 h. Reaction vials also contained a 10-µg sea urchin DNA filter or a 1-µg pSC101 DNA filter where appropriate. Filters were batch-washed sequentially in 1 l of 6×SSC, 2×SSC and 2×SSC containing pancreatic RNase (10 µg ml^{-1}), dried and counted in a liquid scintillation spectrometer[17]. In these conditions ^{3}H-histone mRNA does not hybridise to pSC101 DNA and pSC101 cRNA does not hybridise to sea urchin DNA. A filter containing 10 µg of *M. lysodeikticus* DNA was present in each vial as a blank and its counts (usually less than 50 c.p.m.) were subtracted from experimental values. Since the lysis procedure used recovers various proportions of plasmid DNA and *E. coli* chromosomal DNA, the amount of plasmid DNA on each filter was determined from the ratio of hybridisation of pSC101 ^{3}H-cRNA to pSC101 DNA and to the bacterial lysate DNA. From this value we calculated the c.p.m. of ^{3}H-mRNA hybridising per 1 µg of plasmid DNA shown in the Table. CCC–DNA plasmid was purified from subcultures at each step for comparison and was hybridised simultaneously with ^{3}H-labelled histone mRNA essentially as described above. The purification steps are described in the text. Bacteria from the initial culture of *L. pictus* DNA-pSC101 transformants (line 1) were diluted serially seven times (3:1 dilutions) in an 8×48 well grid made in sterile microtitre plates. The initial inoculum in the top well of each of the 48 columns was 10^{4} bacteria. The wells were then filled with L broth (0.25 ml) containing tetracycline (10 µg ml^{-1}) and incubated in a humidified atmosphere to prevent evaporation. After 24 h of incubation, several wells in the last two rows still had no growth suggesting that limit dilution had been reached. Aliquots taken serially from each of the 48 wells of each row were pooled. The eight resulting pools were cultured individually in 100 ml of L broth overnight and plasmid DNA was isolated as described above. The 48 wells in the last positive row (line 2) were arranged in a 6×8 grid and pools were made from each of the eight rows and six columns. DNA isolated from cultures of the first row (line 3) and the fourth column (line 4) hybridised to histone mRNA identifying the intercept well having bacteria containing histone DNA. DNA isolated from a culture of this single well (line 5) was markedly enriched for histone DNA sequences. Cells from this well were streaked on nutrient agar; 96 individual colonies were picked and placed in the wells of an 8×12 microtitre plate. After overnight incubation, aliquots from the wells of each row of this plate were pooled and cultured. DNA isolated from each culture was tested for the presence of histone DNA. One row (line 7) was positive and cells from the 12 wells in this row were individually cultured. DNA isolated from cultures of each well in the row was tested by RNA–DNA hybridisation. Cells from one well (line 8) gave a positive signal and was designated plasmid *L. pictus* 1 (pLp1).

rDNA[8,9]. The DNA was then used to transform calcium chloride-treated cells of the restriction-deficient *E. coli* strain HB101. After the 42 °C heat-pulse DNA uptake step of the transformation procedure, bacteria were incubated in L broth and diluted into the same medium containing tetracycline (Tc, 25 µg ml^{-1}), as described previously[19,20]. The cells were grown to stationary phase, and DNA isolated from the unfractionated heterogeneous population of Tc-resistant bacteria by detergent (Brij-58) lysis[21] was extracted with a phenol–chloroform mixture, loaded on to nitrocellulose membrane filters[17,22] and hybridised with the homologous radioactive *L. pictus* or *S. purpuratus* histone mRNA (Table 1). Covalently closed circular (CCC) DNA isolated[23] from the same bacterial culture was analysed by hybridisation for comparison. The specificity and authenticity of the histone mRNA used as a probe for the presence of histone DNA sequences has been documented previously[16,24].

We estimated that histone DNA sequences would be present initially in one of every 2,000–3,000 cells of the heterogeneous Tc-resistant transformed bacterial population. This estimate was derived from the observed frequency of chimaera formation when similar conditions were used for ligation of *Eco*RI-generated fragments of *X. laevis* rDNA to the pSC101 plasmid[8] and the fraction (0.25%) of sea urchin DNA estimated to represent histone genes[25]. Hybridisation of DNA isolated from the total transformed bacterial population with the tritium-labelled histone mRNA probe (Table 1, line 1) indicated that DNA sequences complementary to the RNA were detectable.

The specific bacterial cells containing histone DNA were cloned selectively from the heterogeneous population of Tc-resistant transformants by an adaptation of a method for subculture cloning of bacteria[26]. 10^{4} cells were placed in each of the 48 wells in the top rows of microtitre plates and diluted serially to a final concentration of approximately four cells per well. From the calculated frequency of histone DNA in the plasmid population, we estimated that by the fifth dilution, bacteria in only one of the 48 wells in the entire row would contain histone genes, which would be present in one of every 60 cells in that well. DNA isolated from a pooled cell population obtained from each row was tested for the presence of cloned histone DNA by hybridisation with ^{3}H-labelled sea urchin mRNA. We then used DNA–RNA hybridisation to identify the final dilution that contained a positive signal, to find the 'positive' well(s) in that row, and to purify further those specific bacteria that contained DNA complementary to the histone mRNA (see legend to Table 1).

Several experiments of this general design were carried out using eukaryotic DNA from *L. pictus* or *S. purpuratus*; Table 1 shows the results of a typical experiment. The amount of histone mRNA hybridising per µg of plasmid DNA increased at each stage of the isolation procedure, as transformants containing histone DNA were purified from the background of plasmids which lack histone DNA. The 48 wells of the row containing the last dilution that yielded a positive hybridisation signal (Table 1, line 2) were rearranged into a grid, and aliquots from the wells of each row and each column of this grid were pooled. We cultured the resulting pools of bacteria and then tested DNA isolated from each culture for the presence of nucleotide sequences which hybridised to histone mRNA. The resulting hybridisation pattern (Table 1, lines 3 and 4) facilitated identification of an intercept well (Table 1, line 5) containing bacteria which carried histone DNA plasmid chimaeras. Cells from this

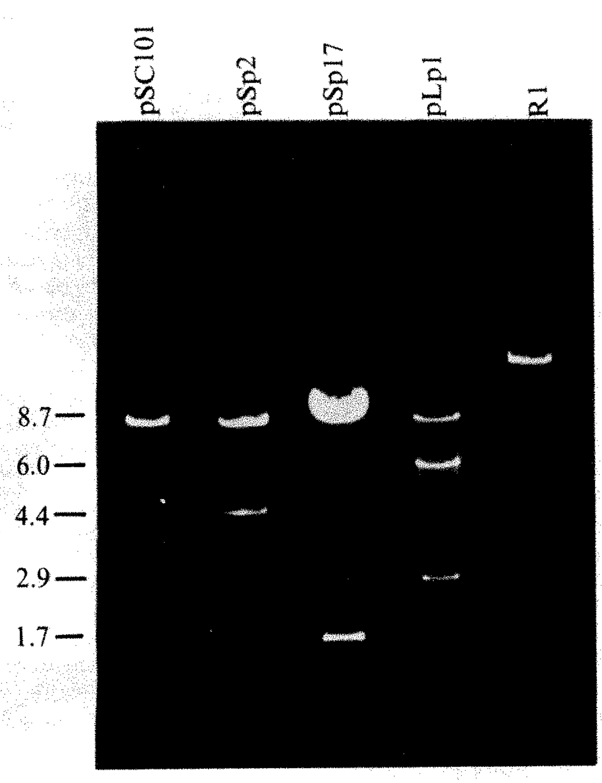

Fig. 1 Agarose gel electrophoresis of *Eco*RI-cleaved DNA of pLp1, pSp2, and pSp17 as well as pSC101 and R1 plasmids. The conditions used for *Eco*RI cleavage and for electrophoresis in 0.8% agarose gels have been described[9]. The sizes of fragments of plasmid chimaeras are expressed in kilobases (kb). Fragment size was calculated from mobility in the gel relative to the mobility of fragments of plasmid R1 DNA cleaved by the *Eco*RI endonuclease[33].

well were streaked on nutrient agar plates, and individual colonies were selected for further analysis by DNA–RNA hybridisation (see legend to Table 1).

This subculture cloning method, which can be carried out using either centrifuged detergent lysates of bacterial cells or purified plasmid CCC–DNA, enables rapid identification of those pools of bacteria containing plasmids that carry specific eukaryotic genes. It is potentially applicable for the isolation of any gene or gene combination which may be present in unknown quantities in a heterogeneous population of DNA, as long as a suitable probe is available. When cleared bacterial cell lysates are used, the procedure can be completed in 7–10 d when one cycle of lysis and hybridisation is performed daily.

In certain experiments, histone genes were first purified 60–70-fold by repeated centrifugation in caesium chloride–actinomycin D gradients (refs 6 and 26, and compare Fig. 4*a*) or alternatively, those plasmids containing randomly inserted fragments of sea urchin DNA were selected from a heterogeneous DNA population by repeated cycles of transformation and sucrose gradient centrifugation[9] before cloning of the specific histone genes. When such initial purification was carried out, hybridisation results suggested that more than 1 in 1,000 plasmids contained histone genes. In these instances, we used a variation of our subculture cloning method; 100–150 bacteria obtained from cultures yielding a positive signal were cultured to stationary phase in each of ten flasks. Aliquots were set aside, and DNA isolated from the remaining bacteria in each flask was assayed by DNA–RNA hybridisation for the presence of histone genes. The cells from flasks which had proved positive were subcultured by regrowth in ten additional flasks (10–15 cells per flask) and tested once more for DNA which hybridised with histone mRNA. Bacteria from flasks showing a positive 'signal' were streaked on agar plates; randomly selected colonies from each plate were cultured separately in liquid media, and the clones containing histone genes were identified by DNA–RNA hybridisation.

These various methods permitted the isolation of bacterial clones which contained *L. pictus* or *S. purpuratus* DNA complementary to histone mRNA. One clone containing *L. pictus* DNA (Table 1, line 8) and two containing *S. purpuratus* DNA were selected for further study.

Characterisation of plasmid chimaeras

Covalently closed circular plasmid DNA isolated from these three clones was cleaved by the *Eco*RI restriction endonuclease, and examined by agarose gel electrophoresis (Fig. 1). Each

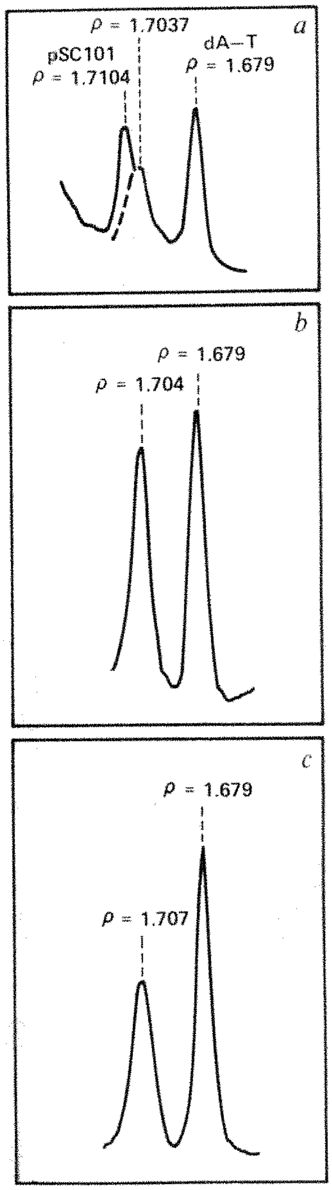

Fig. 2 CsCl gradient centrifugation analysis of plasmid chimaera DNA. *a*, *Eco*RI digest of pLp1 DNA. Separate peaks banding at $\rho = 1.710$ g cm^{-3} (pSC101 fragment) and $\rho = 1.703$ g cm^{-3} (eukaryotic DNA fragment) are seen. The eukaryotic DNA fragments of the pSp2 (4.4 kb) (*b*) and pSp17 (1.7 kb) (*c*) plasmids were separated from the psSC101 DNA fragments by agarose gel electrophoresis[9] of *Eco*RI-cleaved plasmid DNA before centrifugation. Gel slices containing ethidium bromide-stained DNA fragments were excised and crushed, and the DNA was eluted at 37 °C for 2 h in 10 mM Tris-HCl 1 mM EDTA, *p*H 8.0. Recovery was 60%. The buoyant densities of the eukaryotic DNA fragments and of the dA–T marker ($\rho = 1.679$ g cm^{-3}) are shown. The buoyant density we observed for unfractionated *L. pictus* and *S. purpuratus* DNA preparations (not shown) was $\rho = 1.697$ g cm^{-3}. Conditions for centrifugation have been described[23].

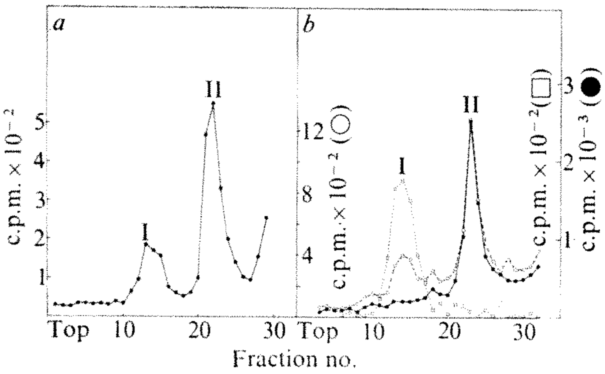

Fig. 3 Sucrose density gradient sedimentation analysis of *Eco*RI-cleaved pSp17 DNA. ^{3}H-thymidine-labelled[19] or unlabelled CCC pSp17 DNA was incubated with *Eco*RI restriction endounclease in the conditions described in the legend for Fig. 1. After heat inactivation of the enzyme at 65 °C for 5 min, the lysates were layered directly over 5–20% sucrose gradients in 1 N NaCl, 0.01 M Tris, 1 mM EDTA and centrifuged at 50,000 r.p.m. for 3.5 h at 22 °C in a Beckman SW 50.1 rotor. Six drop fractions were collected by piercing the tube at the bottom and pumping the contents through an ISCO fractionator. *a*, After centrifugation, *Eco*RI digested ^{3}H-thymidine-labelled pSp17 DNA was collected directly on to Whatman glass fibre filters in scintillation vials, dried and counted in Liquiflor in a Beckman liquid scintillation spectrometer. Two peaks of radioactivity (I and II) were found. *b*, 100 μg of pSp17 DNA was digested with *Eco*RI, divided, and centrifuged on two sucrose gradients with the gradient in (*a*). The collected fractions were divided and plated on each of two 13-mm nitrocellulose membrane filters as described in the legend to Table 1. The dried filters were numbered and cut in half. Each set of split filters was hybridised in 1 ml of 6×SSC-50% formamide containing various radiolabelled RNAs: ○, cRNA synthesised by *E. coli* RNA polymerase with pSp17 DNA as template; ●, cRNA with pSC101 DNA as template; □, sea urchin histone mRNA. The hybridisation peaks I and II correspond to the radioactively labelled DNA peaks I and II in (*a*).

plasmid contained at least one DNA fragment in addition to the 8.7 kilobase (kb) DNA species characteristic of the pSC101 plasmid. The *L. pictus* plasmid chimaera (pLp1) contained two additional DNA fragments with calculated lengths of 6.0 kb and 2.4 kb. Two distinct types of clones containing *S. purpuratus* DNA were observed; one (pSp2) contained a DNA fragment 4.4 kb long (pSp2), whereas the other contained a 1.7 kb DNA fragment (pSp17).

Caesium chloride centrifugation analysis of *Eco*RI-treated pLp1, pSp2, and pSp17 DNA (Fig. 2) indicated that the additional DNA fragments present in all three plasmids have higher densities than the bulk of sea urchin DNA. The observed values are in close agreement with earlier bouyant density estimates for histone genes of sea urchins[6,17,18].

Sucrose gradient centrifugation of *Eco*RI-cleaved, radioactively labelled pSp17 DNA (Fig. 3*a*), demonstrates two endonuclease-generated plasmid fragments (peak I and peak II) with sedimentation properties consistent with the lengths calculated from their mobilities in agarose gels (1.7 kb and 8.7 kb, respectively). Aliquots taken from fractions of a similar sucrose gradient containing unlabelled *Eco*RI cleaved pSp17 DNA were plated on nitrocellulose membranes, and were hybridised in solutions containing either: (1) *S. purpuratus* histone mRNA, (2) complementary RNA (cRNA) synthesised *in vitro* from pSp17 plasmid DNA using the *E. coli* RNA polymerase, or (3) similarly prepared cRNA transcribed from pSC101 plasmid DNA (Fig. 3*b*). Hybridisation with sea urchin histone mRNA was localised to only peak I (the 1.7 kb fragment) of the plasmid chimaera. Both DNA fragments of the pSp17 plasmid contain nucleotide sequences homologous with pSp17 cRNA and as expected, the pSC101 cRNA synthesised *in vitro* hybridised with only the faster sedimenting peak II DNA fragment.

Nine or ten unique RNA species, which code for the several sea urchin histones, are contained in the *S. purpuratus* and *L. pictus* histone mRNA probes used in these experiments[16,24]. To determine whether the two cloned species of eukaryotic DNA were complementary to all or to only some of the RNA sequences in the *S. purpuratus* probe, we hybridised this mRNA with an excess of DNA of each *S. purpuratus* plasmid chimaera. In similar conditions of DNA excess we found that total *S. purpuratus* DNA can anneal with 65–70% of the input RNA (Table 2*a*). In parallel reactions, pSp2 DNA hybridised with 41–44% of the input RNA sequences while pSp17 annealed to 35%. When filters containing DNA from both plasmid chimaeras were included in the same hybridisation mixture, 83% of the input RNA annealed (Table 2*a*). Thus DNA sequences contained in the 1.7 kb and 4.4 kb cloned sea urchin DNA fragments seem to be mostly non-homologous, and together these two cloned DNA fragments probably code for most if not all of the histone mRNA sequences present in *S. purpuratus*. This result also suggests that DNA coding for the several different histones of *S. purpuratus* is clustered within the confines of the two eukaryotic DNA fragments we have cloned and implies that at least some of the repetitive DNA sequences coding for the various *S. purpuratus* histones are intermingled with each other. In addition, the high percentage of histone mRNA sequences homologous with the two cloned eukaryotic DNA fragments in this experiment precludes the possibility that our hybridisation results are attributable to the small fraction of non-histone mRNA (~10%) which routinely contaminates histone mRNA preparations[16,24].

Histone genes in *S. purpuratus* are repeated some 400–500 times[25] and are chromosomally linked into one or a few clusters[17,18]. Since the two *S. purpuratus* chimaeric plasmids

Table 2 Hybridisation data

(*a*) Hybridisation of sea urchin histone mRNA to plasmid chimaera DNA

DNA (μg)	% Hybridised	
S. purpuratus DNA		
10	32	
20	50	
30	72	
40	65	
pSp2 DNA		
10	44	
20	41	
pSp17 DNA		
10	36	
20	35	
pSp2 10	48	83
pSp17 10	35	

(*b*) Hybridisation of ^{3}H-RNA synthesised by *E. coli* minicells containing plasmid chimaeras

DNA on filter	Hybridisation (c.p.m.) of RNA made by: pSC101	pSp17	pSp2
M. lysodeikticus	44	32	37
S. purpuratus	33	637	957
pSC101	8425	7225	2631

a, 850 c.p.m. of trichloroacetic acid precipitable *S. purpuratus* histone mRNA was hybridised with nitrocellulose filters containing the amount of DNA listed. Each reaction was performed in 1 ml of 2×SSC-0.2% phenol at 67 °C for 14 h. The filters were washed as described in Table 1. The c.p.m. hybridised to the individual filters have been corrected for the number of counts hybridising to a filter containing 10 μg of *M. lysodiekticus* DNA (less than 40 c.p.m. in each case).

b, Excess RNA (75×10³ or greater c.p.m.) was placed in 0.4 ml of 6×SSC–50% formamide containing a 10-μg *S. purpuratus* filter, a 10-μg *M. lysodeikticus* filter, and a 1-μg pSC101 filter, and incubated at 52 °C overnight. The filters were washed and treated with RNase as described in the legend to Table 1.

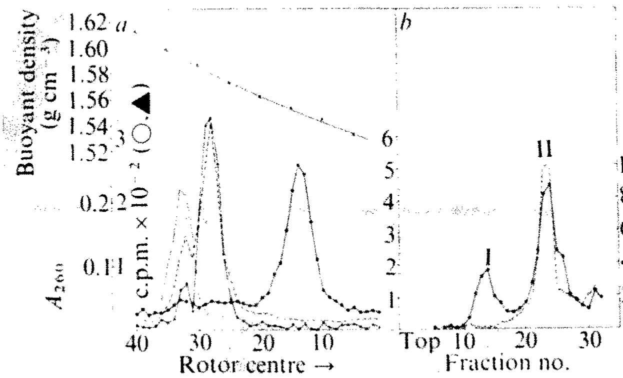

Fig. 4 Hybridisation of RNA transcribed by minicells containing histone DNA plasmid chimaeras. *a*, Hybridisation of minicell transcripts to sea urchin DNA analysed on caesium chloride–actinomycin D buoyant density sedimentation gradients. 1 mg of sea urchin DNA was complexed with an equal weight of actinomycin D (a gift of Merck, Sharpe and Dohme) and centrifuged to equilibrium in CsCl[6,32]. The gradient was fractionated and aliquots of each fraction plated on nitrocellulose filters as described in the legend to Table 1. Multiple sets of filters were made and each hybridised with different radiolabelled RNA preparations. The filters were then batch-washed, treated with RNase A and counted as described in the legend to Table 1. —, A_{260} of main band sea urchin DNA; ●, hybridisation of tritiated rRNA prepared from chick muscle; ○, hybridisation of sea urchin ^{3}H-mRNA; ▲, hybridisation of ^{3}H-uridine minicell RNA from pSp17 plasmid. *b*, Hybridisation of minicell RNA with *Eco*RI digested pSp17 DNA fractionated on a sucrose density gradient. Fractions taken as aliquots from the sucrose gradient shown in Fig. 3*b* were hybridised with 1.2×10^4 c.p.m. of ^{3}H-uridine labelled RNA made by pSp17-containing minicells (●) or with 1.6×10^4 c.p.m. of RNA transcripts produced by pSC101-containing minicells (○). Filters were treated as described above.

collectively contain DNA sequences complementary to all or almost all the histone mRNA sequences, they must contain genes for several different histone proteins. Evidence for a single histone DNA locus obtained from the organisation of histone DNA in *D. melanogaster*[27,28] suggests by analogy that the two *S. purpuratus* fragments are sections of a repeat unit 6 kb long. A unit of this size repeated 470 times[25] would represent approximately 0.3% of the sea urchin genome[29] and corresponds well with previous estimates[24,25]. Our method of selection for histone DNA fragments would not detect histone gene spacer sequences cleaved from coding regions by *Eco*RI restriction endonuclease, and a complete analysis of histone DNA structure may require examination of longer histone DNA fragments.

Transcription in *E. coli* minicells

Plasmid chimaeras containing *X. laevis* rDNA can segregate into *E. coli* minicells[8] when these cells are budded off from the parent *E. coli*[30]. RNA complementary to the eukaryotic gene sequence is synthesised in such minicells[8]. The synthesis of RNA products by eukaryotic genes introduced into bacterial minicells provides an additional parameter for characterising the DNA fragments cloned in the studies reported here. The minicell-producing *E. coli* strain P678-54 was transformed[30] to Tc resistance with pSp2 and pSp17 plasmid DNA. CCC-DNA isolated from purified minicells and treated with the *Eco*RI endonuclease contains agarose gel DNA fragments identical in mobility to those seen with the parent plasmid chimaeras. Minicells containing the plasmids were incubated with ^{3}H-uridine[31], and RNA purified from these cells was hybridised with total sea urchin DNA immobilised on nitrocellulose membranes. The results (Table 2*b*) show that RNA able to anneal with the eukaryotic DNA is synthesised in *E. coli* minicells carrying either of the recombinant plasmids, but not by minicells carrying the pSC101 plasmid alone.

Tritiated RNA isolated from minicells containing the pSp17 plasmid was hybridised also to total sea urchin DNA fractionated by centrifugation in actinomycin D–caesium chloride gradients[6,32]. Our results demonstrate that the RNA transcribed from the sea urchin gene sequences in minicells hybridises entirely with the satellite bands previously identified as histone DNA (Fig. 4*a*). No complementarity of the RNA made in *E. coli* minicells with either main band sequences or ribosomal DNA sequences was observed. An additional hybridisation experiment (Fig. 4*b*) demonstrated that RNA sequences made in *E. coli* minicells containing the pSp17 plasmid chimaera are transcribed from the eukaryotic DNA fragment of the plasmid as well as from the pSC101 segment (a similar result was obtained with the pSp2 plasmid). The amount of hybridisation of minicell RNA with each of the two component fragments of the plasmid chimaera seemed to be roughly proportional to the fragment size.

The subculture cloning procedure described here has enabled identification of individual transformed *E. coli* cells containing histone genes from among a total of 2×10^3–3×10^3 transformants. Other experiments in our laboratories indicate that the method can identify specific gene sequences present at a frequency as low as 10^{-6}. The procedure does not require previous purification of histone DNA sequences or expression of the genes in the bacterium. The technique is potentially applicable for the isolation in *E. coli* of any eukaryotic or prokaryotic DNA fragment for which a specific DNA or RNA probe is available. Moreover, the isolation and purification of groups of genes that function concurrently at different times during the life cycle of complex higher organisms now seems feasible.

Electron microscope analysis of heteroduplex molecules formed between plasmids pSp2 and pSp17 demonstrates no detectable homology of their eukaryotic DNA sequences. In addition, sequences of the eukaryotic fragments from these two plasmids are present within a DNA fragment 6 kb long which is generated by limit digestion of *S. purpuratus* DNA with the restriction endonuclease *Hind* III. Thus the two *Eco*RI derived fragments are in close proximity in the sea urchin chromosomal DNA and the sequences coding for most if not all of the several histone proteins are therefore closely linked within 6,000 base pairs. The details of these experiments will be presented elsewhere.

We thank Jean Lowry for technical assistance, M. Grunstein for sea urchin DNA and S. Panasenko and I. R. Lehman for *E. coli* DNA ligase. This work was supported by the US National Institutes of Health, the National Science Foundation, the American Cancer Society, the National Leukemia Society, the US Veterans Administration and the National Cancer Institute and Medical Research Council of Canada. L.K. was a Scholar of the Leukemia Society of America and is a Medical Investigator of the Howard Hughes Medical Institute.

Received March 13; accepted May 8, 1975.

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letters to nature

Taylor columns in a shear flow and Jupiter's Great Red Spot

THE Great Red Spot (GRS) on Jupiter is not located in a uniform flow but occupies a position at which the zonal wind varies strongly with latitude[1]. The conditions in which Taylor columns form in these circumstances are critically dependent (J. W. Cottrell, unpublished; Woods Hole Oceanographic Institution G.F.D. Summer Programme. Student notes, 1971.) on the sign of the relative vorticity associated with the shear. On Jupiter the GRS is situated at a latitude where conditions are particularly favourable for the formation of a Taylor column, according to this model. Complementary to this theoretical approach we report here some preliminary results of the first experimental investigation into the effect of shear on the formation of a Taylor column.

The suggestion[2] that the GRS could be evidence of a naturally occurring Taylor column—a phenomenon observed in the laboratory when slow, steady flows pass a solid obstacle in a rapidly rotating fluid—has received much attention. Ideas that Jovian atmospheric motions are coupled to topographic features have stimulated several experimental investigations (see, for example, ref. 3) and these have been complemented by related observational data[4] from the terrestrial oceans which confirm the importance of bottom topography in determining certain features of oceanic dynamics.

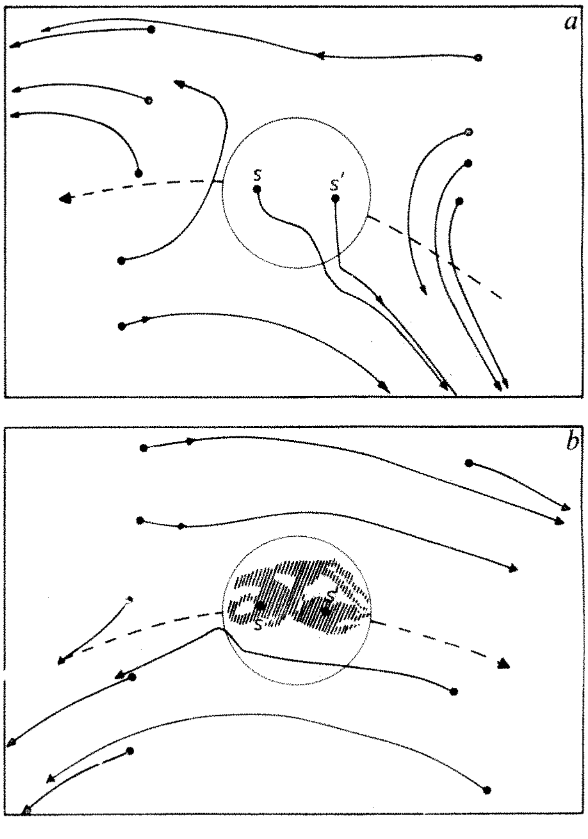
In the case of Jupiter, controversy has arisen over doubts concerning the existence of a solid surface on the planet (the 'topographic feature' is understood to be a mountain or, more probably, an impact crater formed on this surface). The conditions in which 'Taylor columns' form, however, are sufficiently general not to preclude the possibility that they could occur above any large scale irregularity[5], such as a 'floating raft'—an alternative model for GRS proposed on other grounds[6].

The possibility that such a mass of hydrogen-rich solid could float in a fluid layer of hydrogen and helium has received support from laboratory experiments on the phase behaviour of light gas mixtures[7]. Those results indicate that the development of a so-called 'Cartesian diver' model, and the accompanying formation of a Taylor column, are encouragingly similar to several established characteristics[8] of the GRS (change in size, and longitudinal oscillations, for example). Preliminary reports from the Pioneer 10 and 11 missions supposedly add support to the 'long lived storm' hypothesis[9] in spite of earlier criticism[1] of this model. Observations[8] of the anticyclonic circulation of the GRS, on which the terrestrial hurricane analogue is based, refer to the motions in the neighbourhood of the spot and not to motions in its interior (see ref. 1). Indeed, it is noticeable that though the most recent high resolution photographic data[10] reveal some structural details of the GRS, they do not indicate any of the Coriolis effects, such as spiralling motions, usually associated with plan views of terrestrial hurricanes, nor is the shape of the GRS characteristic of such phenomena. Additionally, the 'storm' hypothesis cannot explain the relatively insignificant latitudinal motion of the GRS and its longevity (it has existed for at least 300 yr).

As yet, there does not seem to be sufficient evidence to justify abandoning the Taylor column hypothesis, in some form, in favour of other proposed models (of which the 'long lived storm' seems to be the most plausible); when shear in the zonal flow is taken into consideration the Taylor column hypothesis becomes even more attractive.

Cottrell (unpublished) used the linearised, inviscid equations of motion to consider shear flow past an obstacle, the height of which could be varied. If the sense of ζ_∞ (the upstream relative vorticity associated with the shear) is positive with respect to the basic rotation then, above a critical obstacle height, given by $h = 2\varepsilon\zeta_\infty$, all fluid contacting the imaginary vertical cylinder circumscribing the obstacle is returned, and a

Fig. 1 Plan views of dye-line paths at a height of 3 cm above the top of a cylindrical obstacle of height 1 cm and radius 2.5 cm for: *a*, positive and *b*, negative shear. The sense of the basic rotation is anticlockwise. Dye has been released simultaneously ahead of and behind the obstacle and from two sources, S and S′, within the imaginary vertical cylinder circumscribing the obstacle. The hatched area in *b* represents the accumulated dye from S and S′.



region of closed streamlines forms above the obstacle. Here, ε is the Rossby number of the flow, defined as $\varepsilon = \zeta_\infty/2\Omega$, where Ω is the basic rotation rate. This is equivalent to the critical height derived[2] for uniform flow. In the case of negative ζ_∞, however, all streamlines are closed for any finite value of h, and within the circumscribing cylinder the streamlines are elliptical and aligned with the direction of flow. In this circumstance, the area of the region of closed streamlines above the obstacle (the Taylor column) is directly proportional to the height of the obstacle.

The precise definition of a Taylor column has caused a good deal of confusion[11]: as described mathematically by the Taylor–Proudman theorem[12,13] they do not exist in nature, nor indeed in the laboratory and much effort has gone into determining the detailed structure of the real flow above a slowly moving obstacle in a rotating fluid. Though to the first order the fluid is trapped in the imaginary vertical cylinder circumscribing the obstacle, it is not stagnant, and secondary flows, associated with transport of fluid through the column 'walls', do exist. Here, we find it unnecessary to formulate a numerical criterion for the formation of a Taylor column based on the magnitudes of secondary flows. Instead, we are concerned in a more qualitative way with an obstacle's steering effect[11] on the flow at heights well above the obstacle, and the degree of trapping of fluid above it.

Our apparatus, described in detail elsewhere[14], consists of a rotating cylindrical tank of fluid in which a basic azimuthal flow inversely dependent on the radius of the tank is generated by means of a source–sink arrangement[3]. A cylindrical obstacle (of a dimension typically 1/15 of the tank diameter) is rotated azimuthally through the basic flow at a velocity such that, relative to the obstacle, the flow is forward near the centre of the tank and reversed near the rim. Between these extreme positions there is a monotonic variation in the relative azimuthal velocity. The obstacle is thus situated in a horizontal shear flow, the sense of which can easily be changed by reversing the source–sink pumping arrangement and the sense of rotation of the obstacle. An array of vertical dye-generating electrodes[15] is attached to the obstacle in order to visualise the flow, and a camera fixed to the rotating turntable records the plan view of the flow pattern.

Preliminary qualitative results agree encouragingly with predicted flow patterns (J. W. Cottrell, unpublished) and noticeable differences between situations in which the relative vorticity associated with the horizontal shear is positive or negative with respect to the vorticity of the basic rotation are confirmed (see Fig. 1). Furthermore, variations in obstacle height have a significant effect on the trapping of fluid above the obstacle. Definite Taylor column effects were observed with all cases of negative vorticity flows, though in situations where the relative vorticity was positive only obstacles above a certain size gave rise to flows like those associated with Taylor columns. This result supports qualitatively the theoretical prediction (J. W. Cottrell, unpublished) that in shear flows of negative vorticity Taylor columns can be expected to form in the presence of all obstacles of finite heights, whereas for uniform flow or for positive shear flow a minimum obstacle height is required for Taylor column formation.

As already stated, the GRS occupies one of the few regions of high negative vorticty on Jupiter, so conditions for the formation of a Taylor column at this location are particularly favourable. It would seem that the original Taylor column hypothesis[2], considered in conjunction with the possibility that a hydrogen-rich solid can exist in equilibrium with a hydrogen–helium liquid phase[7] and with the results concerning the effects of horizontal shear, leads to a plausible model for the GRS, in which many of the prominent features (occurrence, shape, uniqueness, short period longitudinal oscillation) are explained. Detailed quantitative experiments on the effect of shear over a wide parameter range are being conducted at present. The possible modifying effect (J. W. Cottrell, unpublished) of the variation with latitude of the local vertical component of the rotation rate (the so-called β effect) are also to be considered in this series of experiments. It is hoped that these quantitative results will enable the parameter range under which Taylor columns form in a shear flow to be established more precisely in order that the extent to which topographic coupling with the Jovian atmosphere, as an explanation of the GRS, can be gauged.

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Received March 18; accepted April 16, 1975.

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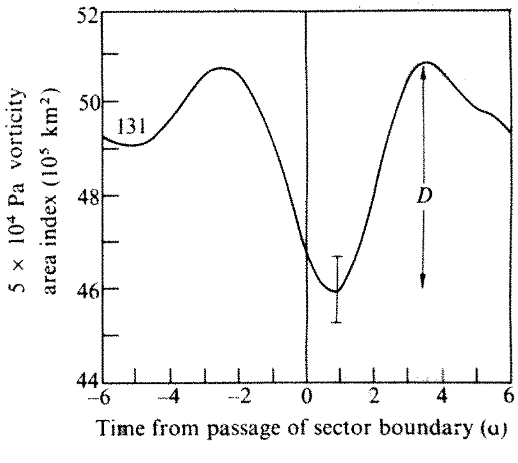
Seasonal variation and magnitude of the solar sector structure–atmospheric vorticity effect

A RELATIONSHIP between the solar sector structure, as swept past the Earth by the solar wind, and terrestrial atmospheric vorticity has been reported by Wilcox *et al.*[1]. The solar sector structure is a large scale property of the Sun that is seen most readily in its magnetic field. As observed by spacecraft magnetometers near the Earth, the extended solar magnetic field typically consists of four sectors within a 27-d synodic solar rotation period. Within each sector the magnetic field is predominantly either towards or away from the Sun. The sectors are separated by thin current sheets that reverse the direction of the field and constitute sector boundaries. Here we report evidence of a seasonal variation in this effect.

In the interval from about two days before a boundary is swept past the Earth by the solar wind to about four days after,

Fig. 1 Average response of the 5×10^4 Pa vorticity area index to the solar magnetic sector structure during the winter months November to March of the years 1963 to 1973. Sector boundaries were carried past the Earth by the solar wind on day zero. The probable error of the mean is indicated with an error bar, and the depth D of the minimum with respect to the average of the two adjacent maxima is indicated.



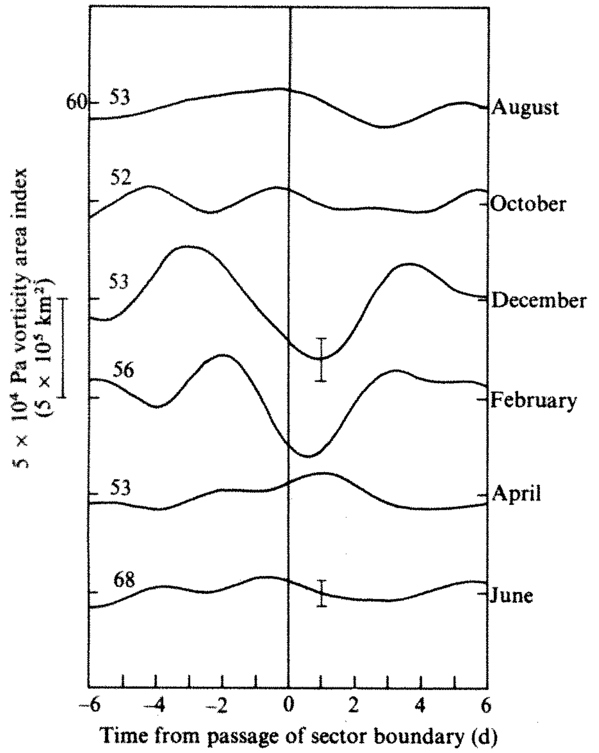


Fig. 2 Seasonal variation of the solar sector–vorticity area index effect shown in Fig. 1. The analysis leading to each curve is the same as the analysis leading to Fig. 1, except that for the curve labelled August, 60 boundary passages in the interval from July 16 to September 15 have been included, with analogous analyses for the other curves. The average value of the index for each curve is indicated with tick marks. Error bars are shown corresponding to the largest and the smallest s.e.m. for one day after the boundary passage. The ordinate range shown for 5×10^5 km² applies to all six curves. The curves have been displaced in the vertical direction for clarity.

the vorticity area index[2] declined by about 10%, reaching a minimum about one day after the boundary. (The vorticity area index is a measure of the size and prominence of all the low pressure troughs in the region of the Northern Hemisphere north of 20°N.) Several properties of the Sun (and, by extension, of the solar wind) vary in an organised manner throughout each sector. The vorticity area index responds to these large scale variations in the sector structure. The precise time at which a boundary is swept past the Earth provides a convenient and well-defined timing marker. A description of the solar sector structure, its extension past the Earth by the solar wind, and the resulting effect on the terrestrial vorticity area index is given elsewhere[3].

The previous analysis[1] has been repeated with the time extended to the interval 1963–73 and the number of cases increased from 54 to 131. We have used the 5×10^4 Pa (500 mbar) vorticity area index of Roberts and Olson[2] with a discriminator setting of 20×10^{-5} s^{-1}. The results are very similar[3] at 3×10^4 Pa and at 5×10^4 Pa; here we use 5×10^4 Pa because the observations are more homogeneous. The index has been filtered to remove variations corresponding to periods greater than 13 d and less than 3 d, to remove the effects of seasonal variations and of short term fluctuations. The results (Fig. 1) show the average variation of the vorticity area index as the solar sector structure is carried past the Earth by the solar wind. The results are very similar to those reported previously[1] although the number of sector boundaries analysed has increased from 54 to 131.

The magnitude of the effect is substantial. The depth D of the minimum as defined in Fig. 1 is about 4.6×10^5 km² which is approximately 10% of the average value of the index. The area of the low pressure troughs where the vorticity is greater than 20×10^{-5} s^{-1} in the winter Northern Hemisphere decreases on the average by about 10% with a minimum one day after a sector boundary passage.

Alternatively, we may discuss the amplitude of the effect in terms of the standard deviation of the data as compared with the depth D of the minimum. The standard deviation is 12.3×10^5 km², so that the depth D (4.6×10^5 km²) of the solar sector-related effect is about one-third of the deviations.

To investigate the seasonal variation of the effect, the analysis leading to Fig. 1 has been repeated using two-month intervals (Fig. 2). The top curve labelled August includes the interval from July 16 to September 15, with analogous intervals for the other curves. The effect shown in Fig. 1 is clearly seen in the winter curves of Fig. 2 labelled December and February, but is not seen in the curves corresponding to the other seasons. A similar curve (not shown) computed for January was very similar to the curves for December and for February, suggesting that the effect is of approximately constant average amplitude through the winter. The fact that this effect is prominent only in winter may be related to the suggestion by King[4] that the Earth's magnetic field may influence, through some unknown mechanism, the behaviour of the lower atmosphere at high latitudes in winter.

In Fig. 2 the curves labelled December and February represent separate analyses of 53 and 56 sector boundary passages. The similarity of each of these separate curves to the result shown in Fig. 1 is further evidence for the reality of the effect.

This work was supported in part by the Office of Naval Research, by NASA and by the Atmospheric Sciences Section of the NSF.

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Received February 28; accepted April 29, 1975.

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Distribution function for random distribution of spheres

THE statistical problems associated with a distribution of spheres in space have received considerable attention[1-3], particularly in the limiting cases of very small volume concentrations and very large ones (close to maximum packing). The intermediate range of finite concentration is not as well covered, and this range is considered here.

The difficulties connected with the definition of a random distribution of spheres have been considered elsewhere[1,2] and here the following construction will be used. Let φ be a given volume concentration and N the number of spheres per unit volume; the radius a of each sphere is given by $\varphi = (4/3)\pi Na^3$. Using the convention that the position of a sphere is specified by the position of its centre, one chooses a point P_1 at random in a unit volume and places a sphere there. Then a point P_2 is chosen at random and the distance P_1P_2 is computed. If this distance is greater than $2a$, a sphere is placed there; otherwise the point is rejected. Then a point P_3 is chosen and the same test applied to P_1P_2 and P_2P_3. The procedure stops when N spheres are embedded in the unit volume.

Buevich[5] has proposed a distribution function for a random distribution of spheres:

$$P(i;V) = C_i^n(1-s)^{n-i}s^i, \qquad i \leqslant n \qquad (1)$$

where $P(i;V)$ is the probability that the set of measure V contains i particles; n is the maximum number of particles which may be embedded in V; $s = \varphi/\varphi_m$, where φ_m is the volume fraction corresponding to the maximum packing ($\varphi_m = 0.907$ in two dimensions and $\varphi_m = 0.74$ in three dimensions) and C_n^m is the binomial coefficient.

Equation (1) follows from the assumptions that n is proportional to V, and a 'chaos' hypothesis in the form

$$P(m;\ U+V) = \sum_{i=0}^{m} P(i;U)\,P(m-i;V)$$
$$\text{for } U \times V = \varphi.$$

These assumptions are simpler and more natural than those used in ref. 5, but a difficulty remains concerning the first assumption. There exist sets of an arbitrary measure in which no sphere can be embedded and so the first assumption implies restrictions on the type of sets considered. What these restrictions should be is not clear.

I have been interested (in the context of the theory of suspensions) in the distribution of nearest neighbours in a random distribution and I have conducted numerical experiments to check equation (1) in this context. The nearest neighbour distribution (three-dimensional case) associated with equation (1) is

$$f(R) = -32\pi nR^2\ln(1-s).(1-s)^{(32/3)\pi n(R^3-a^3)} \qquad (2)$$

where $n = \varphi_m/((4/3)\pi a^3)$. The Poisson distribution function and its related nearest neighbour distribution function are contained in equations (1) and (2) as the limit $\varphi = 0$ (when the spheres become points).

A distribution of points was generated as described above and the deviations of the experimental results for this distribution from the values predicted by the Poisson distribution function were obtained and used as references for the work on finite spheres. Similarly the deviations of the experimental and theoretical nearest neighbour distributions were computed.

Distributions of finite spheres were then generated and expressions (1) and (2) were checked against the numerically calculated functions. When computing the distribution functions for the experiments, the spheres close to the walls of the cube were ignored so as to avoid possible boundary influences. The experiments were carried out in both two and three dimensions, the maximum concentrations being respectively 0.39 and 0.22 ($N = 490$ and 725). Attempts to generate greater concentrations were stopped when 50 consecutive points had been rejected.

The deviations between the experimental values and those predicted by equations (1) and (2) were, over the whole range of concentration obtained, of the same order as the reference deviations. These results can be taken as experimental verification of equations (1) and (2).

For a mixture of spheres of two radii a_1 and a_2 the form of equation (1) remains unaltered except for n becoming

$$n = (3/4\pi)\ \varphi_m\ (d_1a_1^3 + d_2a_2^3)^{-1}$$

where the d_i are relative numerical concentrations, so that if n_i is the number concentration of the i particles, $n = n_1 + n_2$, then $d_i = n_i/n$.

Equation (2) becomes

$$f(R) = \sum_i\sum_j d_i d_j f(R;a_i,a_j)$$

where

$$f(R;b,c) = -32\pi nR^2\ln(1-s).(1-s)^{(4/3)\pi n(8R^3-(b+c)^3)}$$

For a mixture of particles the value of the maximum volume concentration φ_m depends, in general, on the ratio a_2/a_1 and d. But the results obtained in the numerical experiments with the ratio a_2/a_1 between 1 and 2 and for d between 0.1 and 0.9 with a constant value of $\varphi_m(= 0.74)$ are in as good agreement with theory, as in the case of uniform spheres. This indicates that the changes of φ_m in respect to the ratios a_2/a_1 and d are relatively small.

So equations (1) and (2) can be safely used at least as approximate expressions in the range covered by numerical experiments. It would be interesting to obtain experimental results for larger concentrations, closer to maximum packing. But it is not clear to me what kind of procedure will be appropriate for generating such concentrations.

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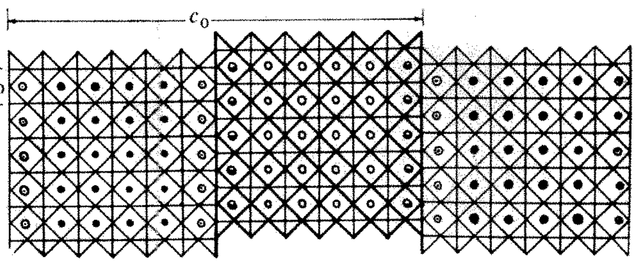
Lattice images of dislocations in the ferroelectric material $Ba_2Bi_4Ti_5O_{18}$

THE compound $Ba_2Bi_4Ti_5O_{18}$ is a ferroelectric[1,2] member of the family of layered bismuth compounds first described by Aurivillius[3]. These compounds are built up by regular intergrowth of perovskite layers with 'Bi_2O_2' sheets. Between the Bi_2O_2 sheets are m layers of corner-shared octahedra containing the B-cations (Ti^{4+}, Nb^{5+} or Ta^{5+}) and ($m-1$) layers of the A-cations (Ba^{2+}, Sr^{2+} or Bi^{3+} and so on) in 12-coordinate interstices. A schematic representation of $Ba_2Bi_4Ti_5O_{18}$, for which $m = 5$, is shown in Fig. 1. As the ferroelectric properties of these materials are connected with their microstructure it is useful to examine them as closely as possible to elucidate the nature of any disorder present. We report here for the first time the direct observation by lattice imaging of dislocation cores in $Ba_2Bi_4Ti_5O_{18}$.

Slightly underfocused lattice images of thin crystals show the actual atomic structure as the projected charge density. By orienting thin crystal fragments so that the electron beam is incident along [100] or [010] of the pseudotetragonal cell, it is possible to obtain lattice images at a resolution of about 3 Å, revealing clearly the positions of the Bi_2O_2 sheets and the perovskite layers. The images show broad, dark lines—the Bi_2O_2 layers, 25 Å apart—with a group of four dark lines

Fig. 1 Schematic projection of $Ba_2Bi_4Ti_5O_{18}$, on (010) of pseudotetragonal cell. Fine grid, B-cations at $y = 0$; heavy grid, B-cations at $y = \frac{1}{2}$; ⊙, Bi at $y = \frac{1}{2}$, in Bi_2O_2 layers; half-filled circle, Bi at $y = 0$ in Bi_2O_2 layers; ●, A-cations at $y = \frac{1}{2}$; ○, A-cations at $y = 0$.



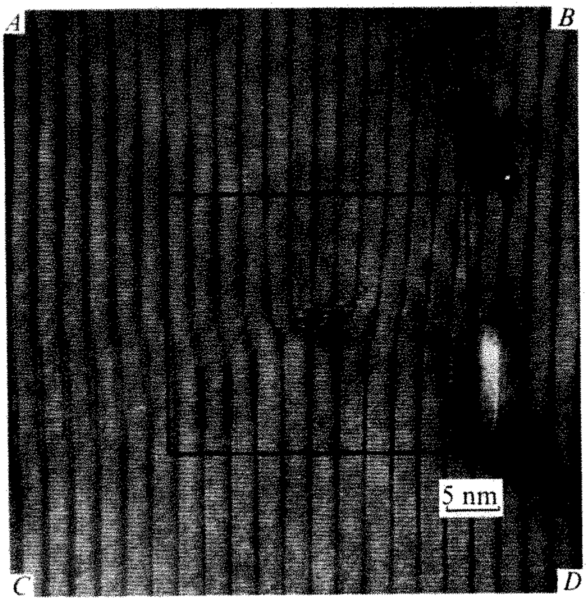


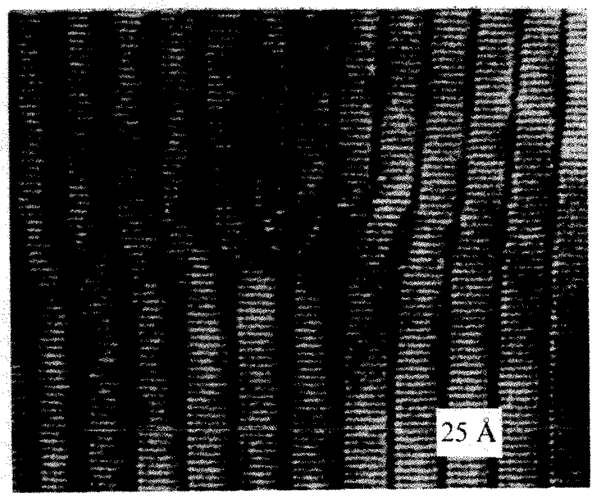
Fig. 2 Lattice image of $Ba_2Bi_4Ti_5O_{18}$, containing a dislocation core.

between them, corresponding to the rows of A-cations (Ba and Bi). These cation sites are sometimes resolved as a square grid, and sometimes as lines perpendicular to the Bi_2O_2 layers—the contrast being critically dependent on defocus, crystal thickness, and so on. Figure 2 shows an image of $Ba_2Bi_4Ti_5O_{18}$ containing a dislocation. There are two extra perovskite slabs and bismuthate layers, corresponding to the insertion of a complete unit-cell of structure. The clarity of the structural detail in the core region (Fig. 3) allows the suggestion that the strain is accommodated in two possible ways.

First, the perovskite layers offer a flexible framework capable of absorbing the compressive strain by distortion of the octahedra. Thus, in the upper part of Fig. 2 there are 23 complete layers (including two extra layers); in the lower part there are only 22 (normal) layers. In the upper part (along *A–B*) the average width is 2.41 nm, in the lower (along *C–D*) the average is 2.51 nm, corresponding to $c_0/2$ (refs 1 and 2).

Second, it is possible for the perovskite layers to slip sideways, in units of one (or more) octahedra. This will shift the Bi_2O_2 layers by about 0.4 nm, as shown in Fig. 4. When the A-cations (Ba^{2+} or Bi^{3+}) are resolved parallel to these layers, the jogging of the Bi_2O_2 layers is clearly visible. Most of the A-cation layers are continuous through these steps, which are also visible in Fig. 3.

Fig. 3 Enlargement of the area enclosed by rectangle in Fig. 2.



The lattice images showed that the crystals contained a very large number of such dislocations, involving one (or more) extra complete layer, although the crystals had been well annealed. This finding may be related to the relatively low Curie temperature for this material (598 K).

The structures of this family of compounds may be described as perovskite lamellae which are in antiphase relationship to each other, and separated by Bi_2O_2 sheets. The faults described may be regarded as dislocations in the Bi_2O_2 superstructure, imposed on the perovskite sublattice. In order to preserve the antiphase relationship across Bi_2O_2 layers, two extra, complete layers are required in a basal dislocation, as in Fig. 2. In a few cases, only one extra layer is observed; this necessarily involves a dislocation in the perovskite substructure itself. A more detailed analysis of these dislocation cores will be published elsewhere.

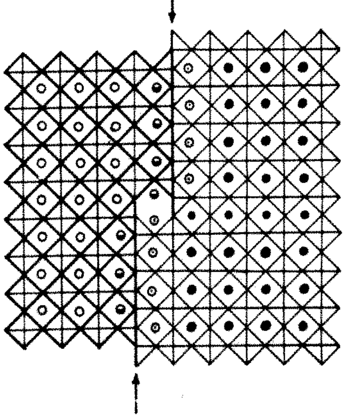


Fig. 4 Idealised drawing of unit shift of 0.4 nm in the Bi_2O_2 layer (indicated by arrows). Symbols as in Fig. 1.

We thank the Science Research Council for support.

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Quality of vacuum-deposited films

THE temperature T_s at which the substrate is maintained during deposition is perhaps the most critical parameter determining the structure and properties of thin solid films produced by the very important techniques of vacuum evaporation and sputtering[1]. Apart from situations when T_s becomes so great as to approach the melting point of the film[2] or to cause complete re-evaporation, the reported variation in properties

Table 1 A summary of substrate temperatures for a variety of vacuum-deposited materials at which optimum properties related to film smoothness/quality have been reported. The optimum temperature T_o is expressed as fractions of the melting point (T_m) and the boiling point (T_b). The vapour pressures at the optima are all calculated to be at least several orders of magnitude below typical evaporator pressures.

Material	Property	Optimum temperature T_o(K)	T_o/T_m (refs for T_m 6, 24, 25)	T_o/T_b	Reference for T_o	Reference for T_b
Anthracene	Smoothness	213	0.44	0.35	—	6
Phthalocyanine	Smoothness	285	—	0.30–0.36	—	*
ZnO	Smoothness, light scattering	768–793	0.34–0.35	0.34–0.36	4	9
Se	Electrophotographic performance	333	0.68	0.35	3, 5	6
ZnTe	Electrical conduction, optical absorption	653	0.43	0.38–0.41	10	11–13
ZnSe	Electro-optic effect	623–643	~0.35	0.34–0.38	14	13, 15–17
PbTe	Hall mobility	523	0.44	0.34	18	19†
CdS	Charge mobility	533	—	0.35	20	21, 22

*Lower limit by equating vapour pressure to that of CuPe (ref. 7); upper limit by including effects of heats of sublimation[8].

†Also refs cited in ref. 19, neglecting the two extreme values.

as a function of T_s is almost always monotonic. Some properties of a few substances have been shown to be optimal at specified T_s and the smoothness of some epitaxial films have been observed to go through a maximum, but only in the case of poly-crystalline metals does there seem to have been any attempt to systematise these observations.

We report a very clear cut structural singularity in certain organic materials as T_s is varied and show that these and practically all other reported results relating optimum properties of evaporated non-metallic films to substrate temperature occur with T_s very near a constant fraction (0.34) of the boiling point of the evaporated materials. This raises the possibility that a very wide range of materials may show optimum properties near this temperature ratio.

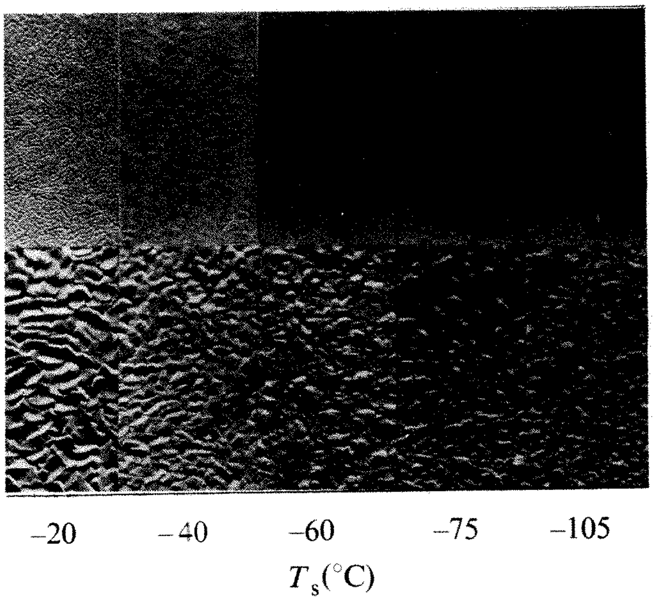
Our measurements have been principally concerned with anthracene films, typically 0.5–1.0 µm thick, which have been evaporated on to thin layers of oxidised aluminium on glass substrates at pressures of approximately 4×10^{-6} mmHg. The anthracene structure is not, however, greatly altered when deposited on other aluminised materials such as mica and melinex and on different evaporated metals. Furthermore, it is not particularly sensitive to the rate of evaporation which has been varied between 15 and 300 nm min^{-1}. Scanning electron micrographs of typical structures obtained at different T_s are shown in Fig. 1. The comparatively smooth structure at −60 °C is clearly not part of the normal monotonic variation in crystallite size. Experiments using graded temperature substrates have shown that the optimum temperature range is quite narrow, in our case being within five degrees on either side of −60 °C.

The electrical properties of the anthracene films and their degree of sensitivity to the optimum substrate temperature will be discussed elsewhere. We do report here some interesting electroabsorption data for our samples. Figure 2 shows the change in transmitted light intensity $\Delta I/I$ arising from the application of an electric field as a function of photon energy for a freshly prepared anthracene film evaporated on to a −80 °C substrate (for details see ref. 3.). The two distinct peaks (*A* and *B*) that are observed at approximately 430 nm and 393 nm, respectively, are present irrespective of the substrate temperature. Single crystal anthracene exhibits an electroabsorption peak at the latter value (G.G.R., unpublished) and consequently we associate peak *B* with a crystalline arrangement of anthracene molecules. Peak *A* is probably more closely linked with absorption processes involving randomly oriented anthracene molecules. The magnitude of this peak for a constant electric field is a strong function of the substrate temperature. The insert in Fig. 2 illustrates how the ratio of the height of peak *A* to the height of peak *B* (which is relatively insensitive to T_s) varies with T_s. The rapid change in the region −50 °C to −70 °C provides strong indirect evidence that a molecular rearrangement occurs near the optimum substrate temperature.

We have obtained similar optima in metal-free phthalocyanine films deposited at 10 °C (which no doubt partly explains why this material is well known to form good films on room temperature substrates). A rather similar optimum has been observed[4] in polycrystalline films of evaporated ZnO at 490–520 °C; this temperature range is the most suitable for integrated optics applications, because excessive light scattering occurs from the rough surface of high temperature films or from the many small particles obtained at low temperatures. Structural factors are also connected[3,5] with the well known electrophotographic optimum T_s (60 °C) for evaporated Se films.

These results are listed in Table 1 together with the optima (T_o) expressed as fractions of the melting point (T_m) and

Fig. 1 Scanning electron micrographs of evaporated anthracene deposited on oxidised aluminium on glass substrates. The temperature T_s of the substrate is indicated. The upper electrode was a thin film of gold deposited on to the structure at −40 °C. All the films were deposited at a rate of approximately 30 nm min^{-1}. The magnifications used for the upper and lower sets of photographs were 2 K and 10 K, respectively.



boiling point (T_b). We have also listed other non-metals for which we could find both optima and boiling point or vapour pressure data in the recent literature. We have excluded cases where a specific interaction with the substrate or proximity to a melting point was influencing the optimum. Most of the materials found do not have known boiling points because they dissociate; we have therefore estimated an approximate effective figure by extrapolating vapour pressure data beyond their range of strict validity. Even with this uncertainty the ratios of optimum to boiling point temperatures for almost all the materials we have found, fall within about 10% of 0.34; there is no such correlation with the melting point. For the examples quoted in Table 1 a range of evaporation rates and thicknesses was used. But it may be significant that the example which deviates most from the observed rule is ZnTe which was evaporated at a very fast rate. Accordingly we intend to examine further the influence of evaporation rate and also film thickness on the quality of our vacuum-deposited materials.

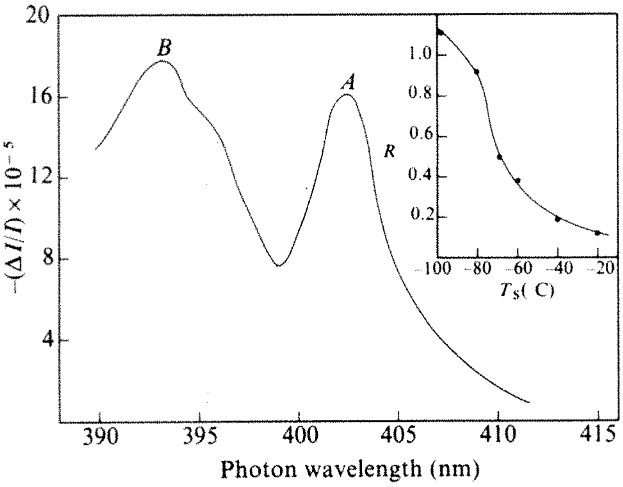


Fig. 2 Fractional change in the transmitted light intensity as a function of photon energy for a freshly prepared anthracene film evaporated on to a substrate held at −80 °C. The insert shows the ratio, *R*, of the electroabsorption peak heights observed at 403 nm and 393 nm as a function of substrate temperature T_S. All data were obtained using an applied electric field value of 5×10^5 V cm^{-1}.

The data of Table 1 provide strong evidence for a close and previously unsuspected relationship between the boiling points and optimum substrate temperatures for a wide range of materials. In most cases it is reported that the surface of the film is smoothest at the optimum temperature. This change in surface quality is probably associated with the occurrence of a structural change in the bulk. Of course several of the film properties involved (such as charge carrier mobility, conductivity and absorption coefficient) are influenced by structural irregularities and therefore a general correlation between these properties and smoothness might be anticipated. It is clear from our electroabsorption studies on anthracene that for this material, the observed smoothness is associated with a transition to a more ordered state. Furthermore it is interesting to note that Genthe and Aldrich[14] have reached a similar conclusion for their results on epitaxial ZnSe films. But, this does not necessarily imply a direct association between the complex mechanism of epitaxial growth and our observed relationship between optimum substrate temperature and boiling point. The optima probably occur because at the given temperatures newly condensed molecules have just enough energy for limited surface diffusion and reduction of disorder to occur but not enough energy for the substantial movement required for such processes as out of plane crystal growth. The constancy of T_o/T_b is probably related to the tendency for the migration energy of an adsorbed molecule to be about one third of its binding energy in the bulk[1]. Our observations suggest that other materials may show optima for $T_s/T_b \sim 0.34$, but there are also materials in which substantial ordering processes can occur without the degree of molecular freedom present at these temperatures; this is presumably the reason, for example, why resistivity and density optima in metals[23] take place at considerably lower values of T_s/T_b. The possibility remains that other optima may be seen at ratios of 0.34 even in these materials.

We thank C. M. Hawkes, B. S. Keating, R. T. Murray and J. Bell for help, and R. A. Hann and C. W. Pitt for discussions.

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Oxidation of nickel by nitric oxide as a new strong oxidant

WE have found that nitric oxide (NO) behaves as a strong oxidant for nickel at high temperatures. Oxidation curves of nickel plates were measured in nitric oxide (700 °C, 10 mmHg) and in oxygen (700 °C, 10 mmHg) by means of a microbalance and the results are shown in Fig. 1. A linear rate law was observed for nitric oxide but a parabolic rate law for oxygen where the rate of oxidation was almost the same as that published[1]. The rate of the oxidation of nickel in nitric oxide was very rapid in comparison with that in oxygen. The X-ray diffraction patterns from the scales formed on both of these samples gave only the patterns of NiO, and the ion microanalysis (IMA) spectra showed no evidence of nitrogen in these scales.

Figure 2 shows the temperature dependence of the rate constant of the oxidation of nickel at 10 mmHg of nitric oxide. The apparent activation energy of the oxidation in nitric oxide was 17.8 kcalorie mol^{-1}, less than that in oxygen (27.7 kcalorie mol^{-1}).

In Fig. 3, the effect of the pressure on the rate constant of the oxidation of nickel in nitric oxide at 700 °C is shown. From this relationship, the reaction order is found to be 0.74 in contrast to the value of 0.16 generally found for the oxidation of nickel in oxygen[2,3]. These findings suggest that the oxidation of nickel in nitric oxide is governed by a surface reaction, whereas that in oxygen is governed by diffusion of the metallic ions through the oxide layers[4].

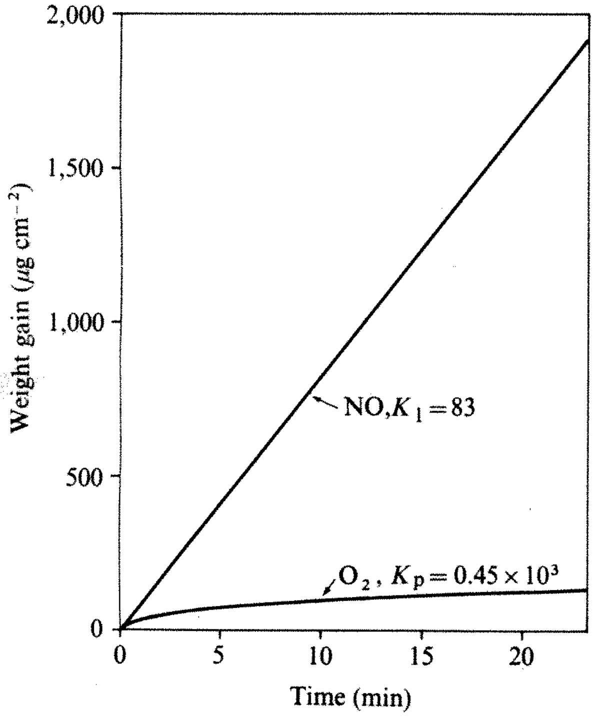
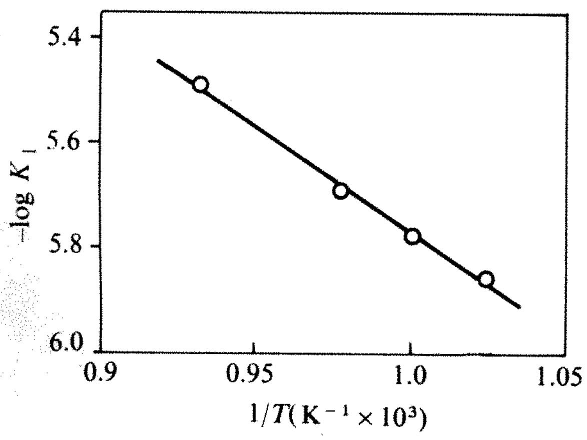


Fig. 1 Oxidation of Ni in nitric oxide or oxygen at 700 °C and 10 mmHg. K_l (μg cm^{-2} min^{-1}), linear rate constant; K_p (μg^2 cm^{-4} min^{-1}), parabolic rate constant.

Fig. 2 The relationship between K_l and temperature (P_{NO} = 10 mmHg). E = 17.8 kcalorie.



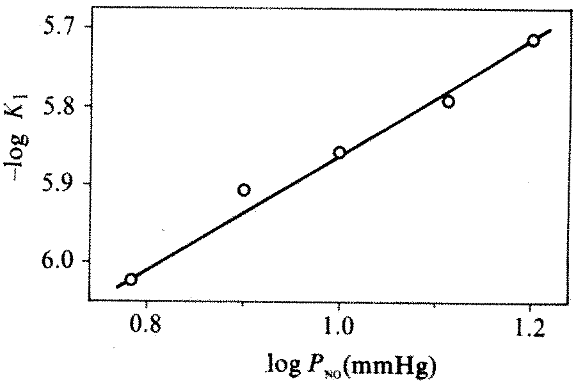


Fig. 3 The relationship between K_l and the pressure of nitric oxide (700 °C). n = 0.74.

We thank Professor Hideo Tamura, Dr Hiroshi Yoneyama and Mr Masaharu Eguchi, of the Faculty of Engineering, Osaka University, for obtaining the IMA spectra.

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Epidemic typhus rickettsiae isolated from flying squirrels

EPIDEMIC typhus is a severe rickettsial infection that classically has involved only man and his body louse. Although the aetiological agent, *Rickettsia prowazekii*, has been isolated from ticks of domestic animals and from blood of livestock in Ethiopia and Egypt[1,2], infection of these unusual hosts and vectors has been thought to be secondary to active dissemination of the louse-borne disease in the human population[3]. Ormsbee concluded that livestock and the equivalent wild species were unlikely to be important in the ecology of epidemic typhus[4]. This disease was last reported in the eastern United States in Philadelphia in 1836 (ref. 5). We have now isolated six strains of *R. prowazekii* from the eastern flying squirrel (*Glaucomys volans volans*). This finding suggests that an extrahuman reservoir of epidemic typhus can exist in a species of wild rodent independent of the recent occurrence of epidemic or sporadic disease.

Serological evidence of this enzootic typhus group infection in flying squirrels was first found near Montpelier, Virginia in 1963. Complement fixation (CF) tests, using species-specific corpuscular antigens prepared from yolk sacs infected with epidemic and murine typhus rickettsiae and *R. canada*, were carried out on specimens collected in 1968. The results showed that, contrary to previous assumption, *R. typhi* was not the causative organism, but that it was either *R. prowazekii* or *R. canada* or another organism antigenically related to both.

CF tests with sera from 30 more squirrels collected near Ashland, Virginia in the spring of 1972 showed that 42% of the animals were infected. During the autumn and winter of

[illegible]

Table 1 Data relevant to isolation of epidemic typhus rickettsiae from flying squirrels from Florida

	Serology IF titre[*] (d after arrival in laboratory)			Results of isolation attempts		
Gv No.	1st serum	2nd serum	3rd serum	Tissues inoculated into	IF titre when killed	Rickettsiae isolated in eggs
F-28	< 10[†](12)	2,560 (19)	2,560 (27)[‡]	*Gv*F-8	2,560 (15)[§]	Yes
F-31	< 10 (12)	640 (19)	640 (28)[‡]	*Gv*F-12	640 (14)	Yes
F-38	< 10 (12)	10 (19)	2,560 (27)[‡]	*Gv*F-16	2,560 (15)	Yes
F-75	< 10 (6)	10 (12)	10 (13)[‡]	*Gv*F-18	2,560 (13)	Yes
F-82	< 10 (6)	10 (12)	10 (13)[‡]	*Gv*F-24	2,560 (13)	Yes
F-134	< 10 (1)	2,560 (8)	—	Eggs	—	Yes

[*] Final titre was the highest dilution of serum that gave 1+ fluorescence on a scale of 1 (minimal) to 4 (maximal) on antigen smears of infected yolk sac suspensions of epidemic typhus rickettsiae standardised so that there were approximately 1,000 organisms per high-power field. Serial fourfold dilutions (1:10–1:2,560) of serum were used. Anti-*Glaucomys volans* (*Gv*) fluorescein isothiocyanate-labelled globulin was prepared from serum from rabbits immunised with flying squirrel serum globulins. When typhus group antibodies were found, either in animals just received or after conversion in the laboratory, in almost every instance the IF titre with *R. prowazekii* was higher than with the *R. typhi* and *R. canada* antigens. *R. rickettsii* and *C. burnetii* antibodies were not found in any of the flying squirrels.

[†] Reciprocal of serum dilution.

[‡] Day animal killed.

[§] Day after inoculation.

1972–73, 20 squirrels were trapped in this area and killed. Attempts to isolate rickettsial agents from suspensions of spleen, liver and kidney inoculated into embryonated hens' eggs and into guinea pigs were negative.

To avoid disturbing the ecological balance in the Ashland area, animals were obtained from a commercial source in Florida. Between December 1973 and July 1974, 215 flying squirrels were received, all recently captured in wooded areas in northern and central Florida. Within a few days after arrival they were bled by the orbital technique[6]. Their sera were tested by indirect immunofluorescence (IF) for antibodies to *R. prowazekii* (Breinl strain), *R. typhi* (Wilmington), *R. canada* (2678), *R. rickettsii* (Sheila Smith) and *Coxiella burnetii* (Henzerling). When first bled, 104 squirrels had typhus group antibodies, that is, their sera had an IF titre of 1:10 or greater. Seronegative and seropositive squirrels were housed in an isolated room in different cages, usually in groups of six. The 111 negative animals were bled again at 6–10-d intervals for 3 weeks and at 2-month intervals thereafter. In 27 of the negative squirrels, seroconversions, indicated by the presence of antibodies in subsequent specimens, were noted and 15 of these animals were killed. A 20% w/v suspension of liver, kidney and spleen homogenised in Snyder I diluent[7] was prepared from each animal. The first 13 suspensions were each inoculated intraperitoneally into a previously confirmed seronegative squirrel, and the other two directly into the yolk sac of 6–8-d-old embryonated eggs (SPAFAS, Inc.). Five of the inoculated squirrels developed typhus group antibodies within 12–14 d; each was killed and a pool of its tissues was inoculated into eggs as previously described. Table 1 shows the recovery of rickettsiae from these animals. Another strain was recovered by direct inoculation of eggs with tissues from a squirrel that seroconverted 8 d after arrival. The other attempt at direct isolation using eggs was unsuccessful. Each strain was reisolated in eggs from samples of the corresponding tissue suspensions that had been stored for 5–6 months. The classical

Table 2 Relationship of rickettsiae isolated from flying squirrels to the members of the typhus group as revealed by CF

	Complement-fixing antibody[*] titre with 4 U of specific rickettsial antigen				
Guinea pig antiserum	*Gv*F-12	*Gv*F-16	*R. prowazekii*	*R. typhi*	*R. canada*
*Gv*F-8	640[†]	640	320	< 160	< 160
*Gv*F-12	640	640	640	< 160	< 160
*Gv*F-16	320	320	320	80	< 80
*Gv*F-18	320	640	320	< 160	< 160
*Gv*F-24	640	640	320	< 160	< 160
*Gv*F-134	320	320	320	< 80	< 80
Epidemic typhus	256	256	256	64	< 64
Murine typhus	64	64	64	256	< 64
R. canada	< 16	< 16	< 16	< 16	64

[*] CF tests were performed according to the LBCF microtitre method[10].

[†] Reciprocal of serum dilution.

Table 3 Relationship of rickettsiae isolated from flying squirrels to the members of the typhus group by toxin neutralisation

	50% Toxin neutralisation titre[*] with rickettsial suspensions			
Antiserum	*Gv*F-16	*R. prowazekii*	*R. typhi*	*R. canada*
*Gv*F-16 guinea pig	407[†]	407	≤11	≤11
Epidemic typhus guinea pig	204	362	16	≤11
Murine typhus guinea pig	45	16	512	≤11
R. canada rabbit	≤11	≤11	≤11	16
Virginia flying squirrels	512	362	≤11	≤11

[*] Twofold dilutions of serum were mixed with 4 mouse LD_{50} of toxin and incubated at room temperature for 1 h. Groups of four mice were injected intravenously with 0.5 ml of the toxin–serum mixture. Deaths were recorded up to 18 h. 50% Serum neutralisation endpoints were determined by the method of Reed and Muench.

[†] Reciprocal of serum dilution.

rickettsial strains were never handled on the same day that isolation procedures were carried out, and all autopsies and processing of tissues and eggs were done in a biohazard laminar flow hood.

The egg 50% lethal dose (LD_{50}) titres of early passage seed suspensions of each of the strains ranged from $10^{3.6}$ to $10^{6.0}$; the 50% infectious dose (ID_{50}) titres, determined by examining stained smears of yolk sacs of embryos surviving for 12 d, were 10–500 times higher.

Adult Swiss mice inoculated intraperitoneally with 10% yolk sac suspensions died within a few hours as a result of rickettsial toxin. Suspensions diluted beyond lethal levels caused no apparent disease, but mouse ID_{50} titres based on serological conversion were essentially the same as those found in eggs. Serial twofold dilutions of suspensions of the different strains inoculated intravenously into mice had toxin LD_{50} titres varying from 1:64 to 1:256.

Soluble complement-fixing antigens[8] prepared with the isolated strains were group-reactive in tests with guinea pig, rabbit and mouse immune sera.

Species-specific corpuscular antigens[9] prepared from two strains titred 1:256–1:320 with homologous antibody. The results of grid-type CF tests with these antigens and the other members of the typhus group and corresponding guinea pig sera are shown in Table 2. Thus the rickettsial agents isolated from flying squirrels were closely related, if not identical to *R. prowazekii*. Toxin neutralisation (TN), another definitive method for species identification, showed that the strains were indistinguishable from *R. prowazekii* (Table 3). The results of the TN test with a pool of sera from animals caught in Virginia that had IF titres of 1:640 and 1:2,560 suggest that the rickettsial agent is enzootic also in Virginia.

We thank Dr D. E. Sonenshine, Old Dominion University, Norfolk, Virginia for specimens supplied in connection with collaborative investigations supported in part by contracts from the Department of the Army and the Food and Drug Administration. We thank Mrs N. T. Stocks and Mr J. W. Humphries for technical support and Mr F. Isreal for assistance in the laboratory.

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Received March 12; accepted April 17, 1975.

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Control of cell-contact sites by cyclic AMP pulses in differentiating *Dictyostelium* cells

SINGLE cells of the slime mould *Dictyostelium discoideum* differentiate after the end of the growth phase of their life cycle into interacting cells which form aggregates by chemotaxis towards a source of cyclic AMP[1,2], with the formation of specific contacts between cells[3–5]. Emergence into the aggregation phase coincides with an increase in the number of specific contact sites (known as contact sites A; ref. 3) on the surface of the cells. The appearance of contact sites A is thus an index of differentiation and we have used it to test the hypothesis that cyclic AMP is a signal for initiating differentiation[6,7], as well as acting as the aggregation signal for differentiated cells.

Cyclic AMP is spontaneously released by *D. discoideum* cells before the development of full aggregation competence. The release of cyclic AMP is pulsatile[8,9] and there is evidence to suggest that the chemoreceptor system underlying aggregation responds to the rapid increase in cyclic AMP concentration with time, rather than to the concentration *per se*[9,10]. We therefore used a pulsatile cyclic AMP signal to stimulate differentiation in stationary phase cells. In contrast to cells harvested at the exponential growth phase, differentiation is blocked in these cells. This block can be bridged by cyclic AMP pulses (Fig. 1). Continuous flow application of cyclic AMP at the same average rate had no significant effect on differentiation. The size of the stimulatory pulses (5 nmol per 10^{10} cells) is much lower than the amplitudes of the periodic cyclic AMP spikes which are spontaneously generated by differentiating cells (50 – 100 nmol per 10^{10} cells). Since it is known, however, that extraneous cyclic AMP pulses can stimulate cyclic AMP release from the cells, so that they are amplified by one to two orders of magnitude[9,11], the effective pulse sizes may be close to each other in both conditions.

The formation of EDTA-stable cell contacts is a specific function of contact sites A. To show that the control of these sites by cyclic AMP pulses is based on their accelerated appearance at the cell surface, rather than on the control of their activity, the antigenic expression of contact sites A at the cell surface was measured by F_{ab} binding. Figure 2 shows enhanced expression and function in response to pulsatile signals.

Contact sites A did form in non-stimulated stationary phase cells, as well as in the same cells exposed to continuous flow of cyclic AMP, but there were fewer and they appeared more slowly (Fig. 2). Cell adhesion measured in the presence of EDTA indicated that these sites, when formed, also become active (Fig. 2). Whereas in exponential phase cells the appearance and activity of contact sites A is largely correlated with the ability of the cells to elongate and to adhere preferentially at their ends, both functions are dissociated in stationary phase cells (compare Figs 1*b* and *c* and 2), indicating strict dependence of cell elongation on stimulation by cyclic AMP pulses. Actin is the predominant protein synthesised in the interphase between growth and aggregation[12]. One can speculate that a high rate of actin synthesis is necessary for the change of cell shape, and that this synthesis is under the control of cyclic AMP pulses.

Exponential phase cells showed only slight acceleration of differentiation, which was advanced by about 1 h in response to pulses of 2×10^{-8} M cyclic AMP per 10 min. In these cells the experimental cyclic AMP pulses on one hand induce premature onset of spontaneous pulsing, but on the other interfere with established oscillations, and the results may critically depend on the balance of these actions. Continuous flow at the same average rate delayed differentiation by 4 h, so that the cells were not fully differentiated after a period of 9 h.

Continuous-flow application of cyclic AMP is known to suppress spontaneous oscillations[10]. The inhibition of cell differentiation by continuous flow suggests that in cells collected at the exponential phase, differentiation is self-stimulated by the periodic pulse generating system, and that the continuous flow interferes with but does not mimic its action on cell differentiation.

If differentiation is self-stimulated by the cyclic AMP which the cells release, it should be delayed by cyclic-AMP phosphodiesterase. To test this hypothesis extracellular phosphodiesterase of *D. purpureum* was used because this enzyme is largely resistant to the phosphodiesterase inhibitor produced by *D. discoideum* cells[6]. Consequently, constant activities could be maintained over the whole period of cell differentiation to aggregation-competence. The strain used was the fast differentiating wild-type clone *M2* grown on bacteria; 10^7 cells ml^{-1} were suspended in 0.017 M phosphate buffer *p*H 6.0. In these conditions, relatively high phosphodiesterase activities of 40–80 nmol min^{-1} ml^{-1} were required to delay cell differentiation; and only in one of three experiments was the delay more than 1 h[13]. (The enzyme activities are those calculated for the experimental conditions; they have to be multiplied by a factor of 5 to get the standard activities measured at 35 °C *p*H 7.4, as published previously[6].) The low efficiency of phosphodiesterase in delaying cell differentiation is easily explained if one imagines that for the cyclic AMP released during a pulse, a half life of about 2 s is sufficient to

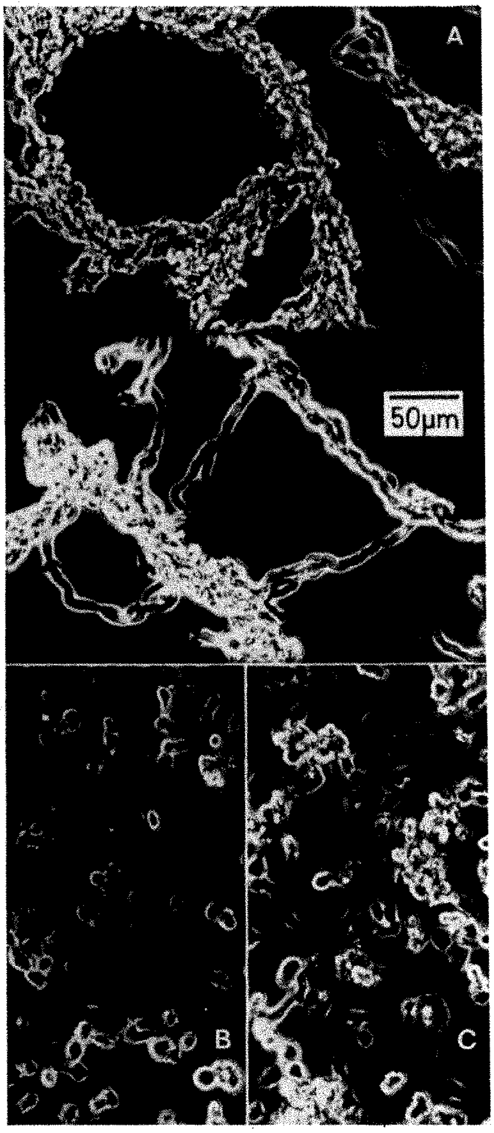

Fig. 1 Effect of cyclic AMP pulses on differentiation of cells collected at the stationary phase. A, Pulse-stimulated cells reached full aggregation-competence within 8 h, as indicated by extensive elongation of the cells, their end-to-end adhesion, and their ability to aggregate into whirl-like aggregates (top) and stream-like assemblages (middle). After steady-flow application of cyclic AMP (C) the cells did not differ from control cells (B). They were not elongated, but tightly sticking to the glass and only partially aggregated into irregular clumps. Cells of the axenic strain Ax-2 of *D. discoideum* were cultivated on nutrient medium supplemented with 1.8% maltose[22], and collected at a density of 2.0×10^7 ml^{-1}, washed and adjusted to 1.0×10^7 ml^{-1} in 0.017 M phosphate buffer *p*H 6.0. 3×10^{-5} M cyclic AMP (A, C) or buffers (B) were fed into 300-ml cell suspension at a constant rate of 0.380 ml h^{-1}, beginning about 40 min after resuspension. In B and C the material was immediately injected into the cell culture, in A it was first collected on a microbalance and dropped into the culture in volumes of approx 40 µl. The mean period of dropping was 6.5 min. Because samples had to be taken for the measurements shown in Fig. 2, the mean amplitude of the pulses increased in A from 4.2 nM cyclic AMP at the beginning to 6.4 nM at the end of the experiment. Accordingly, in C the mean rate of injection increased from 0.63 to 1.0 nmol min^{-1} l^{-1}. Cells were removed from the suspension 8 h after washing, and allowed to aggregate for 2 h in the absence of any stimulation before photographs were taken.

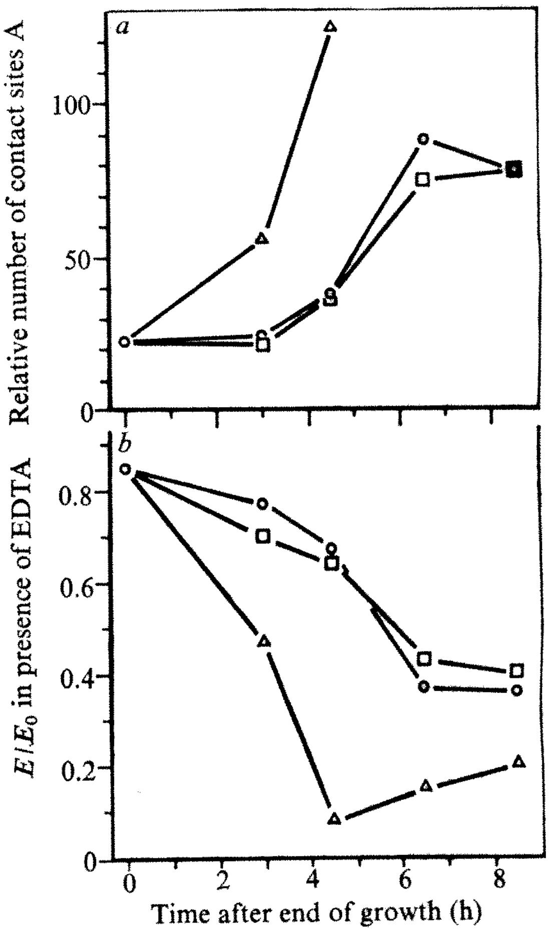

Fig. 2 Exposure of contact sites A at the cell surface (*a*) and EDTA-stable cell adhesion as a measure for the activity of these sites (*b*). △, Cells stimulated by cyclic AMP pulses corresponding to Fig. 1A; ○, control according to Fig. 1B; □, continuous cyclic AMP application as in Fig. 1C. *a*, Contact sites A were measured by absorption of F_{ab} from antiserum directed against aggregation-competent cells. The aggregation-inhibiting activity of the unabsorbed F_{ab} was then retitrated in the presence of 10 mM EDTA using aggregation-competent cells as described in ref. 3. The ordinate value of 100 corresponds to the relative number of contact sites A found in differentiated Ax-2 cells 9 h after collection at the exponential phase. The low background absorbing activity corresponding to a relative number of approximately 20 contact sites A found in non-differentiated cells, is the result of contact sites B which are already present in growth-phase cells and which are not fully inactivated by the EDTA used. Absence of contact sites A from non-differentiated cells has been demonstrated by use of F_{ab} from which the anti-B activity had been removed[3]. *b*, EDTA-stable cell adhesion. Ordinate: *E* refers to light scattering of the test sample, E_0 of an identical single-cell suspension. Low E/E_0 values indicate strong aggregation in the presence of 10 mM EDTA in 0.017 M phosphate *p*H 6.0. Abscissa: time after separation of the cells from the nutrient medium, as outlined in the legend to Fig. 1. All measurements for *a* and *b* were made after washing the cells three times so that the different nucleotide composition of the culture fluids could not directly influence the results.

activate the receptors[9,14] and that the enzyme, by lowering the background level of cyclic AMP, could even increase the sensitivity of cells to pulses. We cannot strictly rule out the possibility, however, that the dependence of cell differentiation on extracellular pulses is a peculiarity of axenically grown cells collected at the stationary phase, whereas in other cells the extracellular loop of the signal system is redundant. In this case, the intracellular oscillation of metabolites could be the key for cell differentiation. During an oscillatory cycle, the intracellular cyclic AMP concentration changes by a factor of 10 with peak values of 20 μM.

It is possible to interpret these results in terms of a receptor system which responds to a rapid increase of cyclic AMP concentration in time. A receptor system of this type would also account for the observation that, on an agar surface, cyclic AMP has to be applied in a spatial gradient to induce cell adhesion[1]. When guided by chemotaxis, the cells migrate towards higher concentrations, so that the cyclic AMP concentration at their surface automatically increases with time.

The response to concentration steps would relate the morphogenetic signal system in *D. discoideum* to other sensory systems exhibiting an 'on'-response. Examples are the phototropic response of *Phycomyces*[15], phototaxis in colonial green algae[16] and chemotaxis in bacteria[17,18]. These results underline the possibility that not only the time pattern of signalling[19], but also the dynamics of the receptor system, could be an important parameter for the action of morphogens in controlling embryogenesis. In relation to neurodevelopment it may be of interest that, in *Dictyostelium*, the formation of adhesion sites operating in specific cell connectivity is facilitated by the action of a low molecular weight transmitter which, released in periodic pulses, passes an intercellular gap. Oscillations of acetylcholine in stimulated electroplax tissue[20] parallel the cyclic AMP oscillations found in *Dictyostelium* and suggest similar control mechanisms[21,23] for the transmitter synthesising enzymes in both systems.

We thank Mr H. Zeller for assistance. The work was supported by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.

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Received February 20; accepted May 7, 1975.

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Secretion of cyclic AMP induced by cyclic AMP in the cellular slime mould *Dictyostelium discoideum*

A STRIKING feature of aggregation in large species of *Dictyostelium* is the appearance of repeated waves of centripetal movement propagated outwards from the centres[1,2]. I accounted for them as follows[3-8]. The cells are not only oriented by the chemotactic agent, acrasin[9], but induced to secrete it; they thus relay the orienting signals. The detailed aggregation pattern, before contact modifies it, records the transmission of these signals. To avoid an infinite regress, some secreted factor must induce its own secretion; this was tentatively assumed to be the attractant itself. There would then be separate thresholds for attraction and secretion. The dual responsiveness develops during the preaggregation phase. Aggregation centres are started by the first cells to secrete signals supraliminal for their neighbours. That separate cells, having oriented to the signal and transmitted it outwards, do not reorient to the signal now coming from behind them and transmit it centripetally, was ascribed to some combination of reduced receptor, motor, and secretory activities, expressed as an absolute or relative refractory period, and to changes in cell shape, spacing, orientation, and polarity, once the cells have responded. The precise signal profile may be significant. After an interval, the cells can transmit a new pulse in any direction. Commonly the same cells as before initiate the later pulses and thus remain dominant, but pulses from other pacemakers can recruit and reorient those cells they reach first. Secreted acrasin is rapidly inactivated enzymically. It was argued that this is functionally useful in maintaining the detectability of the signal even with a single source, and vastly more so with literally millions of sources. Enzymic breakdown could also yield a metabolite for recycling. Later work[10-12] has very largely confirmed this. The attractant in *D. discoideum* is cyclic AMP[13], and the inactivating enzymes phosphodiesterases (PDE)[14-16]. Cyclic AMP pulsed from a microelectrode induces propagated waves of movement[17]. A mathematical model has incorporated most of the above features[18]. The present work demonstrates directly that cyclic AMP does induce aggregating cells to secrete cyclic AMP.

Although cells in suspended culture become aggregation-competent[11], normal aggregation will occur only on a surface, and the standard method for studying the biochemistry of aggregation has been to let the amoebae develop on dialysis membrane, Millipore membrane, or filter paper, and analyse the underlying fluid[4,19]. Cyclic AMP output has been measured at intervals of several hours[20]. Added 10^{-4} M cyclic AMP has been shown to increase Ca^{2+} secretion, measured after 10 min (ref. 21), and most pertinently, actually to decrease cyclic AMP secretion 20-fold, measured after many hours[22].

A quicker method was needed. The cells were made to develop not at an air interface on agar or some fabricated material, but on glass under water. Such cells aggregate normally[9], but the maximum densities are much lower. To compensate, a large perfusion chamber was devised from an optically flat 9-cm Petri dish (Anumbra). The bottom, with a 2-mm diameter hole drilled in its centre, was stood, right way up, inside the inverted lid. The flat glass surfaces were kept 150 µm apart by 3 1-mm^2 Sellotape spacers, and thus enclosed ~0.95 ml of culture fluid, which during perfusion was withdrawn through the central hole and replaced by fluid added at the periphery in the gap between lid and bottom.

D. discoideum NC4 was grown on glucose–peptone agar[9] with *Escherichia coli* at room temperature, and collected near the

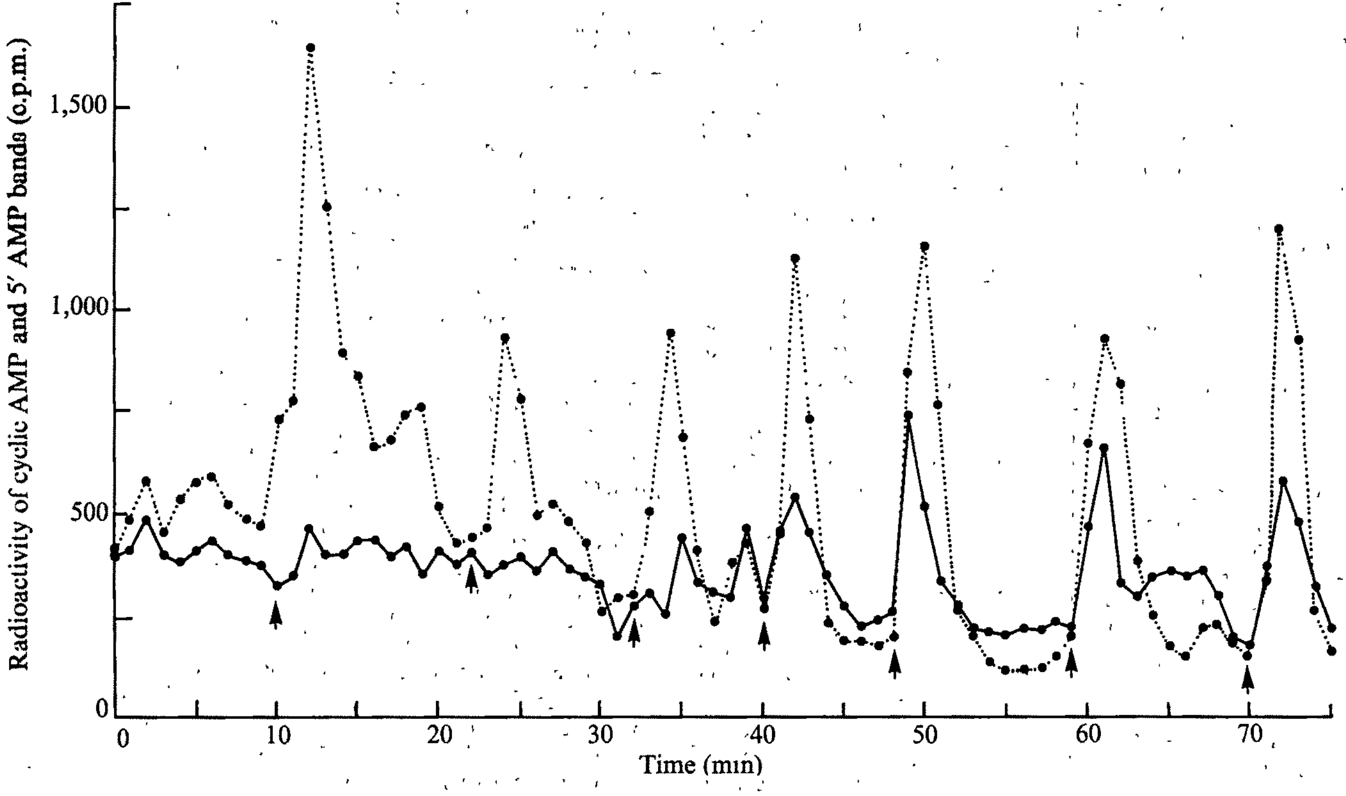

Fig. 1 Radioactivity of the cyclic AMP and 5′-AMP bands from IBAA chromatography of the perfusion fluid of ^{3}H-labelled *D. discoideum* cultures bathed with unlabelled 10^{-6}M cyclic AMP (arrows). Cells deposited at 1,500 mm^{-2} with ^{3}H-adenine and kept stationary. Stippled aggregation after 9 h; cultures then washed and perfused at 20 °C. ——, Cyclic AMP band; ·····, 5′-AMP band. The 5′-AMP band peaks after the first and each subsequent perfusion with cyclic AMP: the cyclic AMP band peaks only after the later perfusions. The apparently anomalous small peak in both bands at 39 min could well have followed the late entry into the chamber of one drop of cyclic AMP temporarily trapped between the rims of the dish.

end of vegetative growth by suspending the cells in ice-cold distilled water. In almost all previous work, such cells have been freed from bacteria by repeated centrifuging[9], but I have found this unnecessary. Instead, the cells were simply allowed to settle in the inverted Petri dish lid, half full of diluted (10 times) Bonner's saline[9], containing 6 mg streptomycin l^{-1}, to give a density of 1,500–2,500 cells mm^{-2}, measured directly with a microscope after spreading. The outermost 5 mm was swept clear of cells. Two washes with saline removed virtually all the bacteria. In most experiments (Fig. 1), the cells were then left in 5 ml of saline containing 100 µCi ^{3}H-8-adenine (17 Ci mol^{-1}) for 8–12 h at room temperature until the majority had entered 'stippled' aggregations: large, rather indefinite fields, in which the cells were elongated and relayed the pulses, but had yet to make extensive contact[7]. This stage was chosen because older cells, in compact streams, tended not to respond to natural external acrasin stimuli[6]. But some cells had already entered more compact aggregations, whereas others were still unaggregated.

The cells were washed three times with saline, left at least 30 min, and washed again. The original bottom of the Petri dish was placed inside the lid, and the surplus fluid sucked up through the central hole until none remained beyond the area of flat, parallel glass. Each minute, 1 ml of fresh saline was added around the periphery in four equidistant aliquots in the period −15 to −5 s, and 1 ml was withdrawn from 0–10 or 0–15 s. At least 20 1-min withdrawals were discarded. Those to be collected were expelled into vials through an AA Millipore filter, which removed any detached cells, and kept on ice. The culture was perfused with 1 ml 1×10^{-6} M cyclic AMP in saline at intervals of 6–12 min. The vials were stood in boiling water for 2 min to inactivate PDE and stored frozen. The contents were dried down and cochromatographed with cyclic AMP, 5′-AMP, ATP, ADP, adenine and adenosine on thin-layer cellulose plates with isobutyric acid–ammonia (0.88)–EDTA (0.1 M)–water, 100:42:1.6:55.8 (IBAA). Bands were scintillation-counted in toluene–Triton X-100 (2:1) containing 0.8% butylphenylbiphenylyloxadiazole.

Peaks of labelled material were found in the band containing cyclic AMP and the band containing 5′-AMP (Fig. 1). But whereas the 5′-AMP band peak appeared after the first and each subsequent perfusion with cyclic AMP, the first appearance of the cyclic AMP band peak was variable. In the Fig. 1 experiment, it appeared clearly only after the fourth cyclic AMP perfusion, doubtfully after the third; in some other experiments, after the second or first.

PDE could readily account for the 5′-AMP peaks accompanying peaks of secreted cyclic AMP. The unaccompanied 5′-AMP peaks may be explained by the presence of more PDE before extensive perfusion, or by slight residual extracellular PDE activity at 0 °C. These explanations seemed challenged by the rather high but fairly constant activity in the cyclic AMP band before its peaks appeared, but were still tenable if this label was not in fact in cyclic AMP. The cyclic AMP band from a continuation of the Fig. 1 experiment was eluted from the IBAA plates with distilled water and electrophoresed on cellulose plates in 50 mM phosphate buffer at *p*H 8. There remained in the cyclic AMP band very marked peaks of cyclic AMP-induced activity, but virtually no detectable activity between peaks. The substantial amount of label that moved towards the cathode was much more constant in successive minutes and unaffected by the applied cyclic AMP (Fig. 2); it may well have been hypoxanthine, which was found to move in almost the same position in these treatments. There was no significant activity elsewhere.

That the label in the cyclic AMP band after electrophoresis was indeed in cyclic AMP was established by re-eluting it with distilled water, pooling the material from several peaks, and incubating with 3′,5′-cyclic nucleotide PDE (Sigma), Tris-buffered at *p*H 7.5 in the presence of Mg^{2+}. After further electrophoresis, as before, all detectable activity was found in the 5′-AMP band and none in the cyclic AMP position.

In later experiments, once the cells had attached to the glass, they were swirled until perfusion time in 8 ml saline and 100 µCi ^{3}H-adenine on a rotary shaker at 50 r.p.m. This not only improved developmental synchrony, but by interfering with the formation of gradients, retarded the appearance of compact aggregations by several hours. Nearly the whole dish became covered with separate, elongated, 'aggregating' cells, mostly lacking any overall orientation: an ideal condition. Also, each collecting vial contained 0.1 ml 0.1 N formic acid, bringing the culture fluid to *p*H 3, at which PDE was inactive. In the Fig. 3 experiment, both cyclic AMP and 5′-AMP bands were eluted from IBAA plates, and separately electrophoresed as before. This did not appreciably purify the 5′-AMP band material. Its identity with 5′-AMP was tested by eluting several 5′-AMP band peaks from IBAA plates with distilled water and incubating the pooled eluate with 5′-ribonucleotide phosphohydrolase (EC 3.1.3.5, Sigma) at *p*H 8, which hydrolyses 5′-AMP to adenosine. After IBAA chromatography, 80% of the label was found in the adenosine band. In the Fig. 3 experiment, cyclic AMP pulses were induced from the first application of cyclic AMP. Also, the 5′ AMP peaks lagged behind the cyclic AMP ones, whereas in earlier experiments they were coincident. This difference may possibly have been caused by hydrolysis in the culture dish no longer being obscured by hydrolysis after collection.

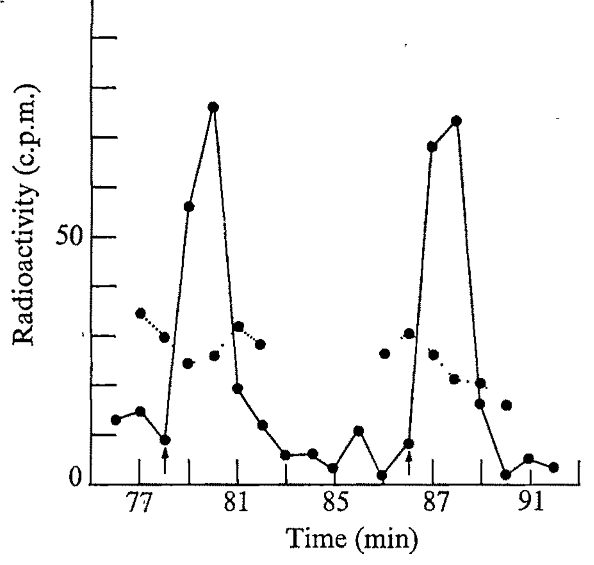

Fig. 2 Later material from experiment in Fig. 1 further purified. 10^{-6} M cyclic AMP added as shown by arrows. Cyclic AMP band from IBAA chromatography eluted with 1 ml distilled water. 0.3 ml eluate electrophoresed in 50 mM phosphate buffer *p*H 8.0. ——, Radioactivity in cyclic AMP band; ·····, in negatively moving material tentatively identified as hypoxanthine.

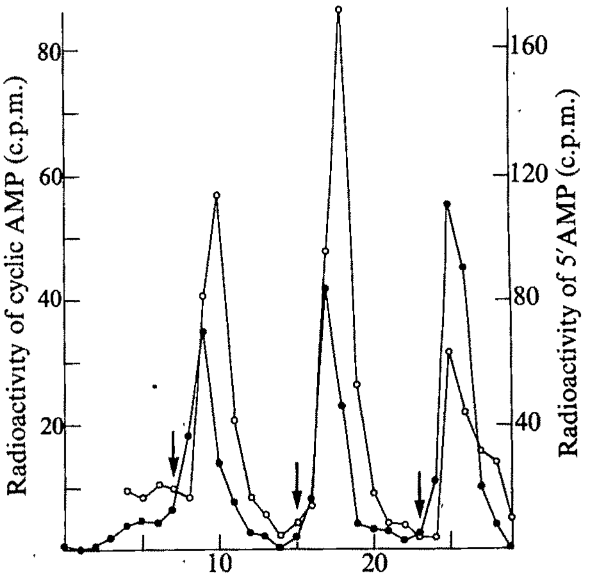

Fig. 3 Cells deposited at 2,500 mm^{-2}, with ^{3}H-adenine, and swirled at 50 r.p.m. for 14 h until stippled aggregation, then washed. Perfused stationary at 20 °C. 10^{-6} M cyclic AMP added as shown by arrows. Each 1 ml collected added to 0.1 ml 0.1 N formic acid. ●, Radioactivity of cyclic AMP; ○, 5′-AMP after elution from IBAA chromatogram and electrophoresis in 50 mm phosphate at *p*H 8. Decomposition during a lengthy delay before the last 8 min of 5′-AMP was processed partly accounts for the low value of the third 5′-AMP peak.

Apart from the actual existence of the peaks, the most striking feature was their extended time course. Certainly the culture chamber allowed appreciable mixing at each perfusion, so that not all the added cyclic AMP was washed out after 1 min; nor was all the label secreted in any 1 min collected at the next withdrawal. Nevertheless, this alone could not readily account for cyclic AMP activity often being, as in Fig. 3, several times greater in the minute following removal of most of the added cyclic AMP. The individual cell responses may have been much shorter, and the lengthy response a cascade triggered by the applied cyclic AMP. Added 1×10^{-5} M, 1×10^{-4} M, and 1×10^{-3} M cyclic AMP, however, induced similar but not quicker responses. If the observed response was indeed a cascade, the secreted agent involved could not have been cyclic AMP. The applied stimulus was, of course, very different from that of normal aggregation, and the continual perfusion, removing both secreted and leaked metabolites, would have tended to interrupt any feedback control loops shortening the response. But conditions were sufficiently normal for the few small aggregations with streams present at the start of the earlier experiments to persist through them. Similar time relationships have recently been found[23] in stirred suspensions of aggregation-competent *D. discoideum* cells. Added cyclic AMP induces two periods of reduced absorbance, the first reaching its minimum after 30 s, the other after 80 s. The slow reaction was thought to be equivalent to the propagation of waves in a cell layer, the fast one to the chemotactic response. There was evidence of a refractory period.

The duration of the transmitted response strongly influences the model of aggregation. An extended mathematical treatment[18] has concluded that the chemotactic signal reaching a cell lasts only 0.2 s, and reversing the sequence of my model[6], that the cell itself signals 15 s later, and only subsequently begins its oriented movement, which lasts 100 s. The calculated brevity of the received signal, actually a consequence of assuming signal release to be durationless, requires the cell to remain chemotactically refractory throughout this whole period. Recent observations[12] indicate that any refractory period of movement in fact lasts less than 12 s, thus again raising the question of why cells do not reorient to cells signalling behind them, albeit that a lengthier refractory period of secretion can prevent the centripetal transmission of pulses. The answer may lie in the profile and duration of the propagated pulse[7], and the effect of the cell's own secretion on its responsiveness to other sources.

That cyclic AMP induces secretion of cyclic AMP contrasts with the observation that it inhibits it. Although the latter result was obtained after exposure for many hours to 10^{-4} M cyclic AMP[22], this negative feedback may nevertheless be of physiological importance, for aggregates of extremely diverse size are often about equally attractive[5,24]. It was concluded[7] that the acrasin output of an aggregate, per cell, is inversely related to aggregate size, and therefore that secretion can be in some way inhibited, possibly, though not necessarily, by acrasin itself, acting extracellularly or intracellularly.

This work was supported by the Science Research Council.

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Received February 6; accepted May 5, 1975.

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Lysis mediated by T cells and restricted by H-2 antigen of target cells infected with vaccinia virus

VARIOUS virus infections lead to the formation of cytotoxic lymphocytes (CL), which are capable of killing virus-infected target cells[1–4]. Specific lysis of target cells infected with ^{51}Cr-labelled vaccinia virus could be observed when investigating the cell-mediated cytotoxic reaction to vaccinia virus[5]; the CL could be characterised as a T cell. The sensitised lymphocytes from C3H mice could only kill syngeneic L929 cells infected with vaccinia virus, whereas lysis by sensitised lymphocytes derived from DBA/2 mice was restricted to the syngeneic infected mastocytoma P815X2 cells. In the lymphocytic choriomeningitis infection the target cell lysis was shown to be restricted by H-2 antigen[6]. We report here experiments with primary fibroblasts of the mouse strains C3H, DBA/2 and the (C3H×DBA/2)F_1 generation were designed to affirm that the effector phase of virus-specific lysis of target cells mediated by T cells is restricted by H-2 antigen even in the vaccinia virus infection. Further experiments with H-2 alloantisera were performed to indicate the close local relationship between H-2 antigens and viral surface antigens.

Primary embryonic fibroblasts of the strains C3H (H-2^{kk}), DBA/2 (H-2^{dd}) and the (C3H×DBA/2)F_1 generation of the two strains (H-2$^{kd\ kd}$) were prepared, infected with vaccinia virus and used as targets for virus-specific cell mediated cytotoxicity. Lymphocytes were collected from mice sensitised 6 d previously. CL from the strains C3H (H-2^{kk}), DBA/2 (H-2^{dd}) and the F_1 (H-2$^{kd\ kd}$) generation were incubated with each type of the three infected target cells. The CL sensitised to vaccinia virus could lyse only syngeneic infected target cells or the semiallogenic infected cells from the F_1 generation, as was the case with permanent cell lines. Accordingly the hybrid CL from the F_1 generation mice were able to kill each of the infected targets (Table 1).

To investigate the local relationship between virus-induced surface antigens and H-2 antigens we tested whether H-2 alloantisera could inhibit the lysis of virus-infected target cells. These sera had no cross reactivity against vaccinia virus. Significant blocking of lysis of infected L929 fibroblasts or mastocytoma P815X2 was observed after incubation of antisera together with CL and infected target cells (Table 2). This effect was restricted by H-2; anti-H-2^{dd} sera could inhibit the virus-specific lysis of infected mastocytoma P815X2 cells, but not of infected L929 fibroblasts by CL from DBA/2 mice or C3H mice, respectively. Blocking could be observed up to a serum dilution of 1:256 when titrations of the H-2 antisera were performed (Fig. 1). Infected F_1 cells were used as targets and incubated with sensitised DBA/2 or C3H lymphocytes with addition of normal, anti-H-2^k or anti-H-2^d serum to determine whether the blocking was effective on the target cell or on the effector cell level (Table 3*a*). In this experiment the action of each CL was inhibited by both of the alloantisera, suggesting that the site of blocking is on the target cell. Secondly, CL from the F_1 generation were prepared and their activity was tested against the H-2^{kk} and H-2^{dd} target cells with the addition of H-2 alloantisera. The results clearly show that blocking was achieved only when an antibody was added, which was directed against H-2 antigens of the target cells (Table 3*b*). Thirdly, we preincubated either the target cell (L929 fibroblasts) or the CL (C3H) with the specific alloantiserum for 30 min at 37 °C, washed them twice with fresh medium and performed the test. As shown in Table 4 preincubation of CL was ineffective, whereas preincubation of the infected target cell caused inhibition of virus-specific lysis.

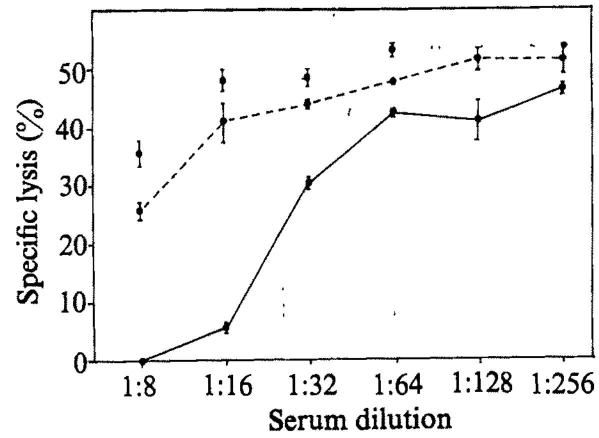

Fig. 1 Blocking effect of H-2 alloantisera on lysis of virus-infected target cells: L929 fibroblasts infected with vaccinia virus. Cytotoxic lymphocytes: spleen cells from C3H mice sensitised to vaccinia virus 6 d previously. Target cells and lymphocytes were incubated together with: ———, anti-H-2^{kk} serum; . . . . , anti-H-2^{dd} serum; - - - -, normal mouse serum.

The results support the concept that the action of virus-specific sensitised CL is restricted by the H-2 gene. Our findings in the vaccinia model are consistent with the observations in the LCM system[6] and similar results were obtained in the ectromelia virus system[4,13]. This restriction is clearly demonstrated for the effector phase *in vitro* and *in vivo*[7], but it may also be operative in the sensitisation phase[8]. Zinkernagel and Doherty[9] pointed out that this restriction does not reflect a physiological interaction with H-2 but rather the immunological surveillance against altered self components.

After addition of H-2 antisera to the *in vitro* test or preincubation of target cells with H-2 antisera significant blocking of target cell lysis can be achieved. This blocking effect is not caused by inhibition of the antigen-specific receptor of the cytotoxic T cell[10–12]. This observation indicates a very close topological relationship between the target antigens and H-2 on the cell surface This target antigen

Table 1 Cytotoxic effect of CL on primary fibroblasts infected with vaccinia virus

Target cell*	Lymphocyte origin	% ^{51}Cr release†	% Specific lysis‡
C3H primary fibroblasts infected with vaccinia virus	Normal C3H	26.9±4.9	—
	Immune C3H	41.3±1.6	14.4
	Normal DBA/2	34.6±0.8	—
	Immune DBA/2	33.8±0.3	—
	Normal F_1 (C3H×DBA/2)	36.6±1.0	—
	Immune F_1 (C3H×DBA/2)	70.0±0.7	34.4
DBA/2 primary fibroblasts infected with vaccinia virus	Normal C3H	55.4±2.9	—
	Immune C3H	37.0±2.0	—
	Normal DBA/2	39.7±2.0	—
	Immune DBA/2	50.2±2.0	10.5
	Normal F_1 (C3H×DBA/2)	39.2±4.4	—
	Immune F_1 (C3H×DBA/2)	64.1±3.4	24.9
(C3H×DBA/2)F_1 primary fibroblasts infected with vaccinia virus	Normal C3H	51.1±1.3	—
	Immune C3H	62.6±2.1	11.5
	Normal DBA/2	46.5±1.1	—
	Immune DBA/2	59.8±1.8	13.3
	Normal F_1 (C3H×DBA/2)	30.4±1.2	—
	Immune F_1 (C3H×DBA/2)	58.8±2.3	28.4

*Primary embryonic fibroblasts from the strains C3H, DBA/2 and the (C3H×DBA/2)F_1 generation were prepared. The second or third passage of these cells was infected with 10 $TCID_{50}$ per cell of vaccinia virus (strain WR) 3 h before the cytotoxic assay. Production of virus-specific surface antigens was assayed by immunofluorescence. Virus-specific membrane fluorescence was visible after 2 h, reaching 80% stained cells after 20 h. CL were prepared[5] from spleens from mice infected intraperitoneally 6 d previously with 1 ml 10^6 $TCID_{50}$ ml^{-1} vaccinia virus. The test was performed using a ratio of 100 lymphocytes per target cell[5]. Incubation was performed over a period of 20 h. Spontaneous ^{51}Cr release from L929 or mastocytoma P815X2 target cells did not usually exceed 30%. Primary fibroblasts were much more sensitive to vaccinia virus infection and often showed high spontaneous release of the label; the addition of normal lymphocytes had a feeding effect and reduced spontaneous cytolysis.

†Each value represents mean±s.e.m. of $n=5$ wells, significance of specific lysis was affirmed by Students' t test ($P<0.01$).

‡Specific lysis was calculated by subtracting ^{51}Cr release in presence of normal lymphocytes from ^{51}Cr release in presence of sensitised cells. Spontaneous release of radioactivity from non-infected fibroblasts did not exceed 35%, CL had no unspecific activities on non-infected cells. Spontaneous release from infected fibroblasts was strain-dependent and varied between 30–50%.

may be either a specifically altered H-2 antigen (altered self), different from viral surface antigens, or it may be a non-specifically altered self plus viral surface antigen[8] and has an unknown topological relationship with both antigens. The blocking activity of H-2 antibodies favours both concepts. There can only be slight antigenic modifications otherwise the H-2 antiserum would no longer bind to the antigen. The blocking of the cytolytic reaction after addition of heterologous vaccinia antiserum[5] without cross reactivity to H-2 antigens seems to be more in favour of the second possibility But it is also not at odds with the first assumption, as steric hindrance of the target antigen cannot be excluded. In preliminary experiments we have found different patterns of H-2 distribution on the surface of

Table 2 Effect of H-2 alloantisera on lysis of virus-infected target cells

Target cell	Lymphocytes	Serum	% ^{51}Cr release	% Specific* lysis	% Blocking
Mastocytoma cells infected with vaccinia virus	—	Normal	29.2±0.2	—	—
	Immune DBA/2	Normal	46.3±0.7	17.1	—
	—	Anti-H-2^{dd}	45.4±0.6	—	—
	Immune DBA/2	Anti-H-2^{dd}	46.5±1.6	0.9	16.2
	—	Anti-H-2^{kk}	36.0±1.0	—	—
	Immune DBA/2	Anti-H-2^{kk}	55.0±1.0	19.0	—
	Normal DBA/2	—	40.8±0.8	—	—
	Immune DBA/2	—	69.9±1.1	29.1	—
L929 cells infected with vaccinia virus	—	Normal	17.7±0.8	—	—
	Immune C3H	Normal	43.7±1.2	26.0	—
	—	Anti-H-2^{dd}	16.6±1.7	—	—
	Immune C3H	Anti-H-2^{dd}	52.3±2.1	25.7	—
	—	Anti-H-2^{kk}	18.2±1.0	—	—
	Immune C3H	Anti-H-2^{kk}	16.7±0.8	—	26.0
	Normal C3H	—	18.0±0.4	—	—
	Immune C3H	—	68.3±5.5	50.3	—

*Specific lysis was calculated by subtraction of lysis in presence of serum from lysis in presence of serum and lymphocytes.

†Blocking effect of H-2 alloantiserum was calculated by subtraction of lysis in presence of H-2 alloantiserum and lymphocytes from lysis in presence of normal serum and lymphocytes.

H-2 alloantisera were obtained from Dr J. G. Ray, Transplantation and Immunology Branch, NIAID, NIH, Bathesda, Maryland. We used as anti-H-2^{kk} serum D-3b, raised in the recipient–donor strains (C3H-H-2°×129) anti-C3H; genotypes were (H-$2^{d/k}$×H-2^{bb}) anti-H-2^{kk}. This serum is cytotoxic for H-2 specificities 11, 23, 25, 52, and also haemagglutinating for specificity 3. As anti-H-2^{dd} serum we used D-31, raised in recipient–donor strains (B10×A) anti-B10.D2; genotypes were: (H-2^{bb}×H-$2^{k/d}$) anti-H-2^{dd}; anti-H-2 specificities were: 31, 34. Details of the raising and testing of these sera are described in the NIH catalogue[14].

Table 3 Site of blocking mediated by H-2 alloantisera

Target cell	Lymphocytes	Serum	% ^{51}Cr release	% Specific lysis	% Blocking
(*a*) (C3H×DNA/2)F$_1$ primary fibroblasts infected with vaccinia virus	—	Normal	47.0±3.0	—	—
	Immune DNA/2	Normal	59.8±3.4	12.8	—
	Immune C3H	Normal	60.4±1.9	13.4	—
	—	Anti-H-2^{dd}	43.2±1.7	—	—
	Immune DBA/2	Anti-H-2^{dd}	44.8±1.7	1.6	11.2
	Immune C3H	Anti-H-2^{dd}	44.2±1.0	1.6	12.4
	—	Anti-H-2^{kk}	50.2±1.9	—	—
	Immune DBA/2	Anti-H-2^{kk}	46.3±2.0	—	12.8
	Immune C3H	Anti-H-2^{kk}	41.1±1.5	—	13.4
F$_1$ primary fibroblasts infected with vaccinia virus	Normal DBA/2	—	46.8±0.9	—	—
	Immune DBA/2	—	59.9±1.8	13.3	—
Uninfected F$_1$ primary fibroblasts	Immune DBA/2	—	47.0±2.0	—	—
	Immune C3H	—	40.7±1.5	—	—
(*b*) L929 cells infected with vaccinia virus	—	Normal	26.6±1.0	—	—
	Immune (DBA/2×C3H)F$_1$	Normal	67.6±2.6	40.9	—
	—	Anti-H-2^{kk}	36.9±3.1	—	—
	Immune (DBA/2×C3H)F$_1$	Anti-H-2^{kk}	31.4±1.1	—	40.9
	—	Anti-H-2^{dd}	50.5±3.6	—	—
	Immune (DBA/2×C3H)F$_1$	Anti-H-2^{dd}	67.6±2.0	41.0	—
	Normal (C3H×DBA/2)F$_1$	—	38.5±3.1	—	—
L929 cells infected with vaccinia virus	Immune (C3H×DBA/2)F$_1$	—	62.0±2.4	23.6	—
Uninfected L929 cells	Immune (C3H×DBA/2)F$_1$	—	18.9±1.8	—	—

Table 4 Effect of preincubating CL or targets with H-2 alloantisera on target lysis cell

Target cell	Lymphocytes	Serum	% ^{51}Cr release	% Specific lysis	% Blocking
L929 cells infected with vaccinia virus	—	Normal	17.9±0.8	—	—
	Immune C3H	Normal	33.8±2.4	15.9	—
	—	Anti-H-2^{kk}	18.6±1.9	—	—
	Immune C3H	Anti-H-2^{kk}	9.7±1.0	—	15.9
	Immune* C3H	Normal	29.4±0.6	11.6	—
	Immune* C3H	Anti-H-2^{kk}	13.1±1.3	—	11.6
	Immune† C3H	Normal	28.8±2.1	10.9	—
	Immune† C3H	Anti-H-2^{kk}	26.6±1.5	8.0	2.9
	Normal C3H	—	22.5±2.0	—	—
L929 cells infected with vaccinia virus	Immune C3H	—	39.4±2.0	16.9	—
Uninfected L929 cells	Immune C3H	—	19.9±1.2	—	—

*Target cells preincubated with serum.
†Lymphocytes preincubated with serum.

infected and non-infected cells by immunofluorescence. In absorption studies vaccinia-infected cells had a reduced capacity to absorb H-2 antisera. Secondly, preincubation of the infected cells with H-2 antisera reduced the vaccinia specific fluorescence. This again suggests a close topological relationship between both antigens.

We thank Ms K. B. Henderson for assistance and Professors R. Thomssen and R. Laufs for discussions. This investigation was supported by the Deutsche Forschungsgemeinschaft.

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Measles virus receptor on human T lymphocytes

ONE of the factors which may determine whether or not a cell is susceptible to a particular virus is the presence in the cell membrane of a receptor or a binding site for the virus[1]. It has been demonstrated that certain viruses, including herpes and myxoviruses, can infect and multiply within lymphocytes[2,3]. Following von Pirquet's original observation[4] it is now well recognised that acute measles infection is associated with depression of cellular (T lymphocyte-dependent) immunity.

More recently, striking cytopathic changes have been described in the thymus of children during and shortly after measles[5]. Furthermore, blood lymphocytes from measles patients form giant cells when cultured *in vitro*[6]. In a cytotoxicity assay devised to study cellular immunity to measles *in vitro* (unpublished), we observed that lymphocytes seemed to cluster around measles-infected fibroblasts in far greater numbers than would be expected if only specifically measles-reactive lymphocytes were involved. We therefore decided to investigate further the nature of this clustering phenomenon. It was found that the majority of human T lymphocytes have surface binding sites for measles virus and, on binding, were cytotoxic to measles-infected cells regardless of the donor's previous experience of measles infection.

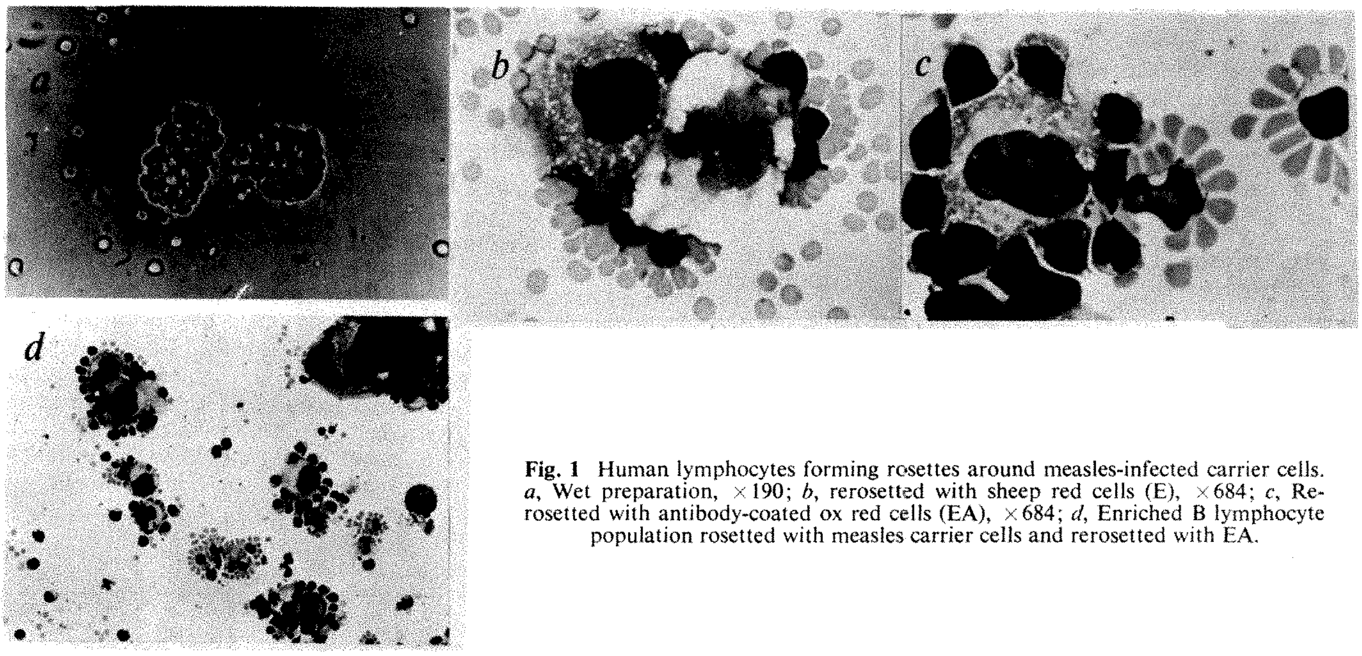

Fig. 1 Human lymphocytes forming rosettes around measles-infected carrier cells. *a*, Wet preparation, ×190; *b*, rerosetted with sheep red cells (E), ×684; *c*, Rerosetted with antibody-coated ox red cells (EA), ×684; *d*, Enriched B lymphocyte population rosetted with measles carrier cells and rerosetted with EA.

A cell line carrying measles virus (Mc-Lu 106) (ref. 7) was used and uninfected control cells (Lu 106) of the same origin.

Binding of measles virus to human lymphocytes was demonstrated by a rosetting technique. Monolayers of infected and uninfected cells were trypsinised and incubated at 37 °C in HEPES buffered medium (HBM) with 5% FCS for a minimum of 2 h. The cells were then washed twice and resuspended in serum-free HBM to a concentration of 10^5 viable cells ml^{-1}. Lymphocytes were obtained from human blood by the Hypaque–Ficoll method[8], washed twice and suspended in serum-free HBM to a concentration of 5×10^5–5×10^6 ml^{-1}. Equal volumes of lymphocytes and measles carrier cells (or Lu 106 control cells) were mixed at ratios from 5:1 to 50:1 in round-bottomed test tubes (LP$_2$, Luckham), centrifuged (300*g*) for 7 min at room temperature and gently resuspended. The cells were examined for rosette formation (LM rosettes) in a haemocytometer, and Giemsa stained cytocentrifuge preparations. Measles carrier cells with three or more lymphocytes attached were counted as rosettes. In optimal conditions 70–90% of the measles-infected carrier cells formed rosettes with human lymphocytes (Fig. 1*a*). Similar proportions of the measles carrier cells showed strong measles-specific membrane fluorescence. Uninfected control cells (Lu 106) showing no measles-specific fluorescence, did not form rosettes. The LM rosettes were also formed by lymphocytes from individuals who had no history of measles as confirmed by absence of measles antibodies. At a low lymphocyte–carrier cell ratio (5:1), 60–70% of human blood lymphocytes were bound by the measles-infected cells. Lymphocytes from several animal species were tested for measles virus receptor. Substantial binding was only observed with monkey, and to a much lesser extent with ox, dog and pig lymphocytes. Other species tested showed minimal or no binding.

Preincubation of the carrier cells with measles antibodies consistently inhibited rosette formation (Table 1). Attempts to block the binding with measles virus were unsuccessful because the lymphocytes were agglutinated by the virus. Trypsinisation of either cell type prevented binding but treatment with neuraminidase and fetuin did not inhibit the rosette formation.

The measles-reactive lymphocytes were further characterised by incubating LM rosettes with sheep erythrocytes (E) and IgG-sensitised ox red cells (EA) (ref. 9). Equal volumes of LM rosettes and 1% red cell suspensions were mixed in LP$_2$ tubes, and centrifuged for 7 min (300*g*) at room temperature and the rosettes examined as before. Most of the carrier cell-attached lymphocytes were firmly surrounded by the sheep red cells (Fig. 1*b*). In contrast, EA did not adhere to bound lymphocytes but formed rosettes with the non-adherent lymphocytes (Fig. 1*c*). Experiments in which the carrier cells were rosetted with B-lymphocyte enriched population, containing approximately 20% E and 54% EA-binding cells, showed a reduced number of LM rosettes, and those formed had few lymphocytes attached. Again, the attached lymphocytes failed to bind EA, whereas the majority of the free lymphocytes did (Fig. 1*d*). Using T-lymphocyte enriched population (less than 5% EA-binding lymphocytes), almost all the lymphocytes were bound and formed strong LM rosettes with the carrier cells. Polyvalent fluorescein-conjugated rabbit anti-human immunoglobulin serum (Hoechst) was used in a direct assay for membrane immunoglobulins. LM rosettes were formed as

Table 1 Inhibition of LM rosettes by pretreatment of carrier cells with measles antibodies

Carrier cells preincubated in	Percentage carrier cells binding lymphocytes*		
	Exp. 1	Exp. 2	Exp. 3
Measles-negative serum	23.3	82.6	87.5
Measles-positive serum	14.0	14.6	61.8

Suspension of carrier cells incubated for 1 h at 37 °C in 1:50 dilution of sera with measles antibody titres of <1:4 and 1:2,048 respectively for haemagglutination inhibition.

*Lymphocyte:carrier cell ratio 10:1.

described above (lymphocyte–carrier cell ratio 10:1) and suspended in an appropriate dilution (1:5 of this antiserum. After 30 min incubation on ice and 3 washes at 4 °C, bound and unbound lymphocytes were examined for membrane fluorescence under the ultraviolet microscope. In three experiments 1.8–4.0% of the carrier cell attached lymphocytes reacted with this antiserum compared with 29–39% of the unbound lymphocytes.

After binding, the lymphocytes were observed to kill measles-infected target cells *in vitro* and this was readily demonstrated by the Trypan blue method. In a chromium release assay, 5×10⁴ carrier cells or uninfected control cells were put into round bottomed test tubes. Human lymphocytes were added to give duplicates of the appropriate lymphocyte target cell ratios (2, 5:1 to 40:1), The final volume of each culture was 1 ml of bicarbonate-buffered 199 medium containing 10% FCS. The cells were centrifuged (300*g*) for 7 min and the pellets gently resuspended before incubation at 37 °C in 5%CO_2 and 95% air. After 1–24 h the tubes were centrifuged and 0.5 ml of medium aspirated for gamma counting.

In this assay, measles-dependent cytotoxic effect could be detected within 2 h, increasing in a linear fashion over 18–24 h. Using 18 h incubation, maximal cytotoxicity was usually observed at a lymphocyte:target cell ratio between 20 and 40. At ratios above 10, however, the measles-dependent effect was sometimes reduced as a result of chromium release from the uninfected control cells. Inhibition of ^{51}Cr release from the measles carrier cells was also occasionally seen at lymphocyte: target cell ratios greater than 20.

Table 2 Toxicity of measles-negative and measles-positive lymphocytes to measles-infected cells

Lymphocyte–target cell ratio	Percentage measles-dependent cytotoxicity*					
	Measles-positive† subjects			Measles-negative‡ subjects		
	Mean	Range	*n*	Mean	Range	*n*
2.5:1	9.3	0–12.8	3	2.2	0–8.2	4
5:1	16.2	2–38.6	5	8.2	1.3–19.5	5
10:1	31.0	6.0–48.2	6	16.3	8.1–21.5	5
20:1	42.8	13.7–75.5	9	22.5	3.8–79.0	10
40:1	53.0	9.1–86.7	5	23.1	6.4–77.7	7

*Obtained by subtracting the percentage cytotoxicity observed in uninfected control cells from that of measles-infected carrier cells.
†Ten subjects tested.
‡Nine subjects tested.

It is evident from Table 2 that lymphocytes from both measles-negative and measles-positive subjects were toxic to measles-infected cells, indicating that the killing did not require the presence of measles-specific lymphocyte clones. Although both groups show considerable scatter, the mean cytotoxicity of lymphocytes from measles-positive subjects was higher than that from those of measles-negative subjects at all lymphocyte: target cell ratios.

We have shown that the majority of human T lymphocytes, and few, if any B lymphocytes, have surface binding sites for measles virus. The reverse has been reported for Epstein–Barr virus, which binds to B but not to T lymphocytes[10]. Our findings also suggest that cell-associated measles virus may activate human lymphocytes nonspecifically.

This work was supported by the Wellcome Foundation. We thank Dr E. Norrby for the cell line carrying measles virus (Mc-Lu 106).

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Effect of concanavalin A and its derivative on the potassium compartmentation of Ehrlich ascites tumour cells

CONCANAVALIN A (con A) has become an interesting probe for the study of dynamic structures of the cell surface[1-3], and of mechanisms of metabolic stimulation such as lymphoid cell transformation[4,5], polymorphonuclear leukocyte respiration[6], and of selective killing of the transformed cells[7]. The critical initial event in these phenomena is the binding of con A to specific receptor sites on the cell surface. One of the most important problems is to determine how interactions of surface receptors with this ligand are linked to the intracellular metabolism. We report here the effect of con A and its succinylated derivative (succinyl con A) on cell agglutination and the K^+-compartmentation of Ehrlich ascites tumour cells (EATC).

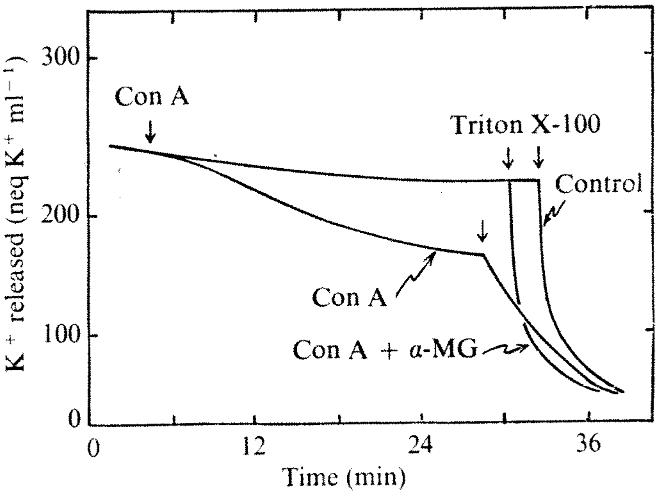


Fig. 1 Effect of con A on the K^+-compartmentation of Ehrlich ascites tumour cells. Cells (2×10^6 ml^{-1}) were incubated with con A (50 μg ml^{-1}) at 25 °C in the presence or absence of 10 mM α-MG. At the end of incubation, Triton X-100 (0.1%) was added to release all K^+ remaining within the cells. Other conditions were described in the text.

EATC were collected from ascites fluid of ddN mice 7 d after intraperitoneal transplantation. Cells were washed 3 times with 0.34 M mannitol containing 10 mM Tris-HCl, *p*H 7.4. Con A was purified from jack bean meal by the method of Agrawal and Goldstein[8]. Succinyl con A, with the same affinity constant for α-methyl-D-glucoside (α-MG) as native con A, was prepared by the method of Gunther *et al.*[9], and further purified by affinity chromatography of Sephadex G-100. They were dissolved in 0.35 M mannitol containing 1 mM $CaCl_2$ and

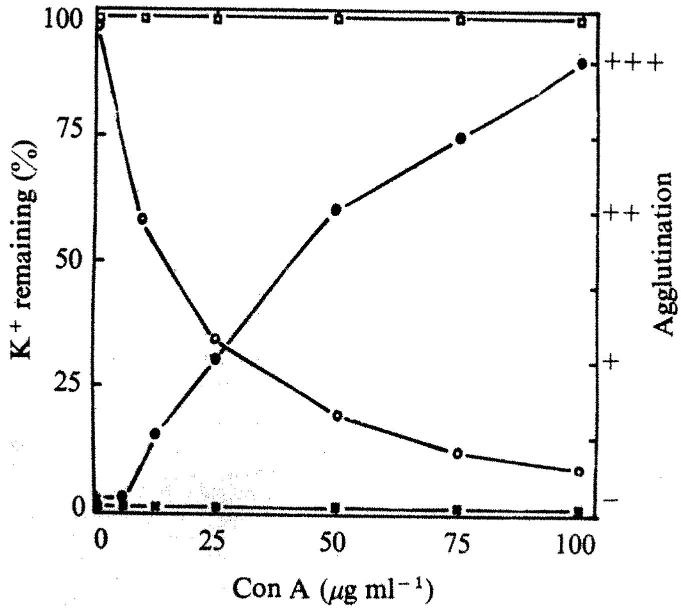


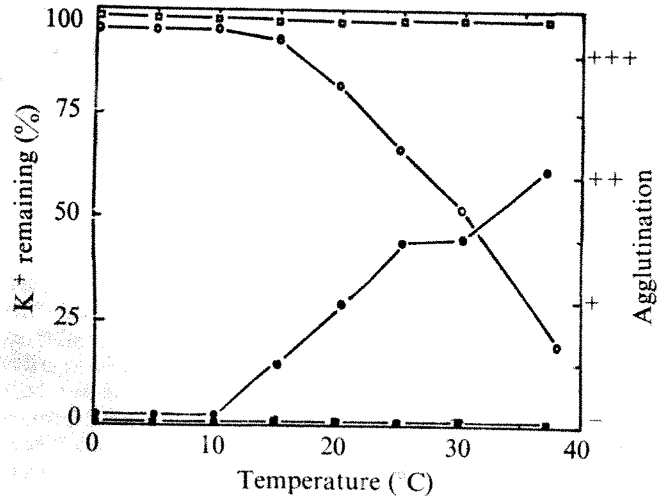
Fig. 2 Effect of con A and succinyl con A on agglutination and K$^+$-compartmentation of Ehrlich ascites tumour cells. Cells (3.4×10^6 ml^{-1}) were incubated with varying concentrations of con A or succinyl con A at 37 °C for 20 min. K$^+$ remaining within the cells was calculated after releasing it from the cells by Triton X-100 (0.1%). Agglutination induced by con A (●), or by succinyl con A (■); K$^+$ remaining within the cells, con A (○), succinyl con A (□).

1 mM MnCl$_2$. All reagents used were of reagent grade. Cell agglutination was scored as described previously[10].

Release of K$^+$ from EATC was measured in the same buffer used for cell washing by K$^+$-electrode (Electronic Instrument Ltd, Model C-KN33C) connected to a pH-meter (Toa Electronic Ltd, Model HM-20B).

Figure 1 shows the effect of con A on the K$^+$-compartmentation of EATC. There is a rapid induction of K$^+$-release from EATC after addition of con A, in agreement with the data reported by Aull and Nachbar[12]. K$^+$-release induced by con A is completely inhibited by α-MG, a haptenic inhibitor of this lectin. The extent of K$^+$-release from EATC increased progressively with increased concentration of con A (Fig. 2). Cell agglutination of EATC was also induced in these conditions. Succinylated derivative of con A, however, induced neither cell agglutination nor K$^+$-release from EATC in the same conditions. Quite a close relationship between the two phenomena was observed as a function of con A concentration. Both of these phenomena induced by con A were temperature dependent, and observed at temperatures above 15 °C (Fig. 3). But, succinyl con A lacks such activity at any temperature tested.

Fig. 3 Effect of temperature on agglutination and K$^+$-compartmentation. Concentration of con A or succinyl con A used was 50 µg ml^{-1}. Other conditions were the same as Fig. 2. Agglutination induced by con A (●), or by succinyl con A (■); K$^+$ remaining within the cells, Con A (○), succinyl con A (□).



Our results suggest that the binding of tetrameric con A to the surface glycoprotein receptors of EATC leads to a change in the properties of the surface membrane, which results in an induction of cell agglutination and K$^+$-release from the cell at temperatures above 15 °C. Edelman suggested that tetrameric con A, and not dimeric succinyl con A, may function to induce cross-linking of surface glycoprotein receptors into micropatches[9], which is inhibited at low temperatures (< 10 °C) because of decreased fluidity of the surface membrane[11].

The same mechanism of con A action that underlies glycoprotein receptor cross-linking may be operating in the changes in K$^+$-compartmentation of EATC. The next objective of our study is to observe how such a change in K$^+$-compartmentation induced by con A affects cellular metabolism.

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Specific alterations of olfactory function in humans with temporal lobe lesions

SUBSTANTIAL anatomical and electrophysiological evidence suggests that human temporal lobe structures are involved in the processing of odour perception. The temporal lobe has both afferent and efferent connections with the olfactory bulb[1,2], the major synaptic junction for primary fibres from the olfactory receptors. Olfactory auras accompany epileptic seizures of temporal lobe origin[3], and electrical stimulation of the uncus and amygdala in conscious patients elicits odour sensations[4]. There are, however, no known quantitative studies of olfactory function in patients with surgically verified temporal lobe lesions. The present findings demonstrate that removal of the temporal lobe increases odour detection thresholds (ODT), whereas odour recognition thresholds (ORT) remain similar to those of subjects without brain damage.

Odour thresholds were determined in patients with surgically defined lesions of the temporal lobe. These subjects had undergone unilateral excision of the anterior segment of the temporal lobe to relieve otherwise intractable epileptic seizures[5]. The lesions extended 5 to 7 cm posterior from the anterior projection of the lobe. This area included the uncus, amygdala

Table 1 Effects of temporal lobe ablation on ODT and ORT

	— log molar concentration of odour (mean ± s.e.m.)					
	Pyridine		Pentyl acetate		Phenethyl alcohol	
	ODT	ORT	ODT	ORT	ODT	ORT
Temporal lobectomy	3.58 ± 0.08*	3.08 ± 0.07	4.58 ± 0.11*	3.91 ± 0.08	5.00 ± 0.13*	3.72 ± 0.09
Control	5.60 ± 0.12	3.50 ± 0.09	7.50 ± 0.13	4.20 ± 0.11	9.90 ± 0.16	5.00 ± 0.13

* Significantly different from control at $P<0.01$.

The forced-choice sniff bottle technique with three stimuli[6] was used to determine ODT and ORT in the test groups. In brief, three 125 ml Erlenmeyer flasks with 18 ml solution were presented in random order to each subject who was then instructed to identify the bottle containing the test odour. Odour determination by random choice is not significant ($P = 0.0004$). In each trial, one flask contained the test odour, pyridine ($CH(CH{\cdot}CH)_2N$; Mallinckrodt, St Louis, Missouri), phenethyl alcohol ($C_6H_5CH_2$–CH_2OH; Matheson, Coleman and Bell, Los Angeles, California) or pentyl acetate ($CH_3CO_2(CH_2)_4CH_3$; Matheson, Coleman and Bell) while the remaining flasks contained the solvent, Millipore-filtered and distilled water. Concentrations of pyridine from 10 M to 10^{-12}M were verified by spectrophotometric measurements at 260 nm to check the accuracy of our dilution technique. As the concentration of the test odour increased in successive trials, a transition occurred where the response of the subject changed from random selection to the correct choice with consistency. Re-testing was carried out about this point. The ODT was defined as the lowest odour concentration at which a subject gave two successive correct responses while giving two consecutive incorrect responses at the next lower concentration. The ORT was determined as follows. Before any trials were given, the subject was shown a short list of adjectives describing four odours. On each of the test trials, the subject was asked to identify its principal characteristics from the list. The ORT was defined as the lowest odour concentration level at which the subject could correctly identify the odour characteristics. Right and left lobectomy patients are grouped together as neither ODT nor ORT of patients with excisions in the right temporal lobe differed significantly from those of left temporal lobectomy patients ($P>0.05$), determined by analyses with the t statistics[15]. The ability of both lobes of the brain to recognise odours is supported by studies in split-brain patients[16].

and portions of the hippocampus, hippocampal gyrus and the fusiform gyrus. All patients were examined at least one year after surgery to avoid possible postoperative effects of oedema on test results. Odour thresholds of 12 right-handed surgical subjects, seven with right and five with left temporal lobectomies, were compared with those of ten control subjects who had no history of damage in the central nervous system.

ODT and ORT levels determined for surgical and control subjects are presented in Table 1. Temporal lobectomy patients had significantly higher ODT levels than controls for all odours tested at $P<0.01$. Conversely, ORTs of the lobectomy patients were not significantly different from the ORT levels of subjects in the control group. The results demonstrate further that the ODT and ORT for each odour were more often at the same concentration level for subjects in the surgical group than were those in the control group ($P<0.001$, χ^2 test).

These data indicate that temporal lobe lesions impair the ability of human subjects to detect, but not to recognise odours at threshold levels. Whereas control subjects can detect an odour at a concentration which is too weak for identification of its characteristics, temporal lobectomy patients can detect an odour only at a concentration level which is strong enough for recognition. This ODT impairment does not seem to result from a deficiency in attention, as intelligence (measured on the Wechsler Scale[7]) and attention (measured by digit span) show no correlation with ODT. In view of these results, it is noteworthy that most of the lobectomy patients rated their olfactory ability as average in their subjective judgment.

Surgical removal of human brain tissue from several neural regions other than the temporal lobe does not elicit consistent alterations in olfactory sensitivity[8]. Tumours in and around the frontal and possibly temporal lobe areas, however, have been reported to induce increments in olfactory thresholds[9]. In studies of the effects of bilateral amygdaloid lesions, transient changes in several olfactory judgments were reported[10]. In-depth EEG evoked responses in the amygdala[10,11] and widespread changes in the unit activity of temporal lobe structures[12] have also been observed in response to olfactory stimuli.

Collectively, these data implicate the human temporal lobe in olfactory information processes, but the nature of that role remains unknown. The potential influences of the ongoing θ rhythm[13] and the other efferent inputs[14] on the transmission of olfactory information to neural areas involved in odour perception should be investigated further. Nevertheless, the present findings indicate that increments in ODT accompany lesions of temporal lobe structures.

We thank the staff and other investigators of UCLA Clinical Neurophysiology Program for their support; and R. J. Pietras and B. M. Wenzel for helpful comments. This research was supported by US Public Health Service grants.

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Muscarinic response to acetylcholine in neuroblastoma × glioma hybrid cells

THE complexity of the central nervous system of vertebrates makes it difficult to identify the cell type responsible for the biochemical or pharmacological effects of drugs observed in the whole tissue. This difficulty does not exist for clonal cell lines derived from tumours of the nervous system. Such lines derived from mouse neuroblastoma C1300 show a number of differentiated functions of neurones[1]. Many of these properties are more strongly expressed in clonal lines of hybrid cells obtained by fusion of mouse neuroblastoma and rat glioma cells (refs 2–4 and B.H., unpublished). In suitable conditions, these undifferentiated cells differentiate by extending long processes[3-5], which contain structures resembling synaptic vesicles[6]. In addition, their membranes become excitable by an electric current (refs 3 and 4 and B.H., unpublished) or by neurotransmitters (refs 3, 4 and 7, and B.H., unpublished). On exposure to prostaglandin E$_1$ (PGE$_1$) hybrid cells strongly increase their intracellular levels of cyclic AMP[8]. This effect of PGE$_1$ is antagonised by α adrenergic agonists[7] and morphine[7,9].

Here we report that the increase in the intracellular concentration of cyclic AMP evoked by PGE$_1$ is also suppressed, if cholinergic agonists like acetylcholine (ACh) or carbamylcholine are present. In neuroblastoma × glioma hybrid cells the effect of the cholinergic agonist is prevented by the blocker of muscarinic receptors, atropine, but not by the inhibitors of nicotinic receptors, D-tubocurarine and α bungarotoxin. Analogous results were obtained with mouse neuroblastoma cells (J.T., unpublished). The neuroblastoma × glioma hybrid line 108CC15 (refs 3 and 4, and B.H., unpublished) and 108CC25 (ref. 7 and R. Heumann, unpublished) are used, since these are found to be the most responsive to PGE$_1$.

Hybrid cells were cultured in plastic dishes (Greiner, 8.5 cm in diameter)[8,10], and incubated for 10 min at 37 °C, at varying concentrations of cholinergic agonists and antagonists in the absence or presence of 3 μmol l^{-1} PGE$_1$, as described previously[9]. The compounds, dissolved in Dulbecco's modified Eagle's medium, were added (50 μl) to 5 ml incubation medium. After the incubation, the cells were assayed for cyclic AMP[10,11]. Analysis of the incubation medium for cyclic AMP that had been released from the cells proved to be unnecessary[9]. The data were referred to cellular protein[10].

The more than 60-fold elevation of the level of cyclic AMP by PGE$_1$ in the hybrid cells is progressively prevented by increasing concentrations of ACh (Fig. 1*a*). The ACh concentration at which 50% inhibition takes place (IC$_{50}$) is 0.1 μmol l^{-1}. No obvious inhibition by ACh was observed when the cells were incubated in the absence of PGE$_1$ (Fig. 1*b*). To classify the cholinergic receptor mediating the effect of ACh, hybrid cells were incubated in the presence of constant concentrations of PGE$_1$, ACh, and eserine, and varying concentrations of blockers of ACh receptors. Whereas the muscarinic antagonist atropine[12] potently blocked (IC$_{50}$ 10 nmol l^{-1}) the inhibitory action of ACh (Fig. 2*a*), only a very slight effect was observed in the presence of the nicotinic antagonist D-tubocurarine[12] (Fig. 2*d*). The snake venom α bungarotoxin (pure toxin, Miami Serpentarium Laboratories), a potent inhibitor of nicotinic action of ACh[13], has no effect when added together with ACh (Fig. 2*c*), and causes only a very slight effect, when added 30 min before incubation with the other drugs (Fig. 2*b*). Even at concentrations up to 0.1 mmol l^{-1} no effect of the toxin could be detected (data not shown). The experiment clearly demonstrates that the ACh receptors of the undifferentiated hybrid cells are of the muscarinic type. Electrophysiological experiments on differentiated hybrid cells had demonstrated ACh receptors which were equally sensitive to atropine and D-tubocurarine and which were blocked by α bungarotoxin (refs 3 and 4, and B.H., unpublished). Experiments are under way to elucidate the nature of this change in receptor specificity.

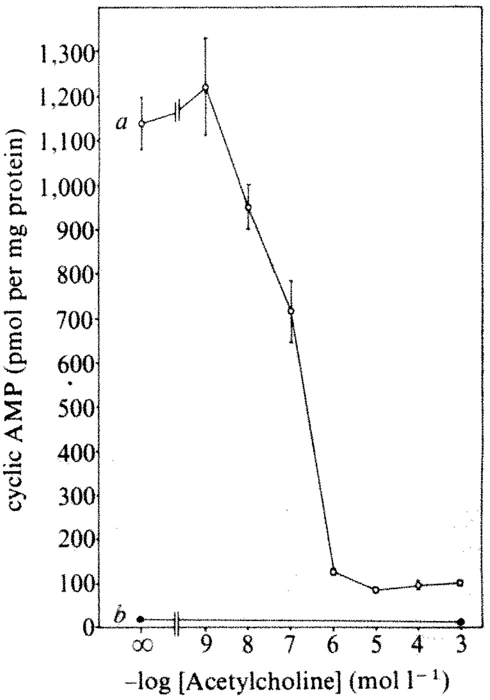

Fig. 1 Inhibition by ACh of the stimulation by PGE$_1$ of cyclic AMP formation in neuroblastoma × glioma hybrid clone 108CC15. At varying concentrations of ACh, the cells were incubated in the presence or absence of 3 μmol l^{-1} PGE$_1$. To prevent breakdown of ACh, the acetylcholinesterase inhibitor eserine (0.1 mmol l^{-1}) was included in all incubations. Subsequently, the cyclic AMP formed was measured (s.d. ± mean of three parallel incubations except (*b*), obtained from single incubations), 2.4 × 10^6 viable cells per plate, passage number 12, viability (exclusion of nigrosin) 96%. The value in the absence of special additions was 16 pmol per mg protein. *a*, Cyclic AMP in the presence of PGE$_1$; *b*, cyclic AMP in the absence of PGE$_1$.

Certain clones of neuroblastoma C1300 show binding constants for α bungarotoxin of about 10 μmol l^{-1}. Muscarinic and nicotinic antagonists are nearly equally effective in preventing the toxin binding[14]. This observation is comparable with that on the differentiated hybrid cells, but not with that on the undifferentiated cells described here.

As with ACh, its analogue carbamylcholine also prevents the increase of the intracellular concentration of cyclic AMP observed in the presence of PGE$_1$ (Table 1). Carbamylcholine also counteracts the elevation of the level of cyclic AMP caused by isobutylmethylxanthine (IBMX), an inhibitor of cyclic nucleotide phosphodiesterase[15,16]. This result suggests that the effect of the cholinergic agonists consists in lowering adenyl cyclase, rather than increasing phosphodiesterase activity. Acetylcholine increases the levels of cyclic GMP in the hybrid cells (R. Gullis, J.T. and B.H., unpublished). Therefore, the prevention by cholinergic agonists of the increase in cyclic AMP concentration in the presence of PGE$_1$

Table 1 Effect of carbamylcholine on the levels of cyclic AMP in hybrid cells treated with PGE$_1$ or IBMX

Addition	Hybrid line	
	108CC15	108CC25
None	10	16
PGE$_1$ (3 μmol l^{-1})	811 ± 109	116 ± 16
Carbamylcholine (0.1 mmol l^{-1})	12 ± 1	11 ± 3
PGE$_1$ (3 μmol l^{-1}) + carbamylcholine (0.1 μmol l^{-1})	168 ± 22	60 ± 3
IBMX (0.5 mmol l^{-1})	41	42
IBMX (0.5 mmol l^{-1}) + carbamylcholine (0.1 mmol l^{-1})	24	23 ± 1

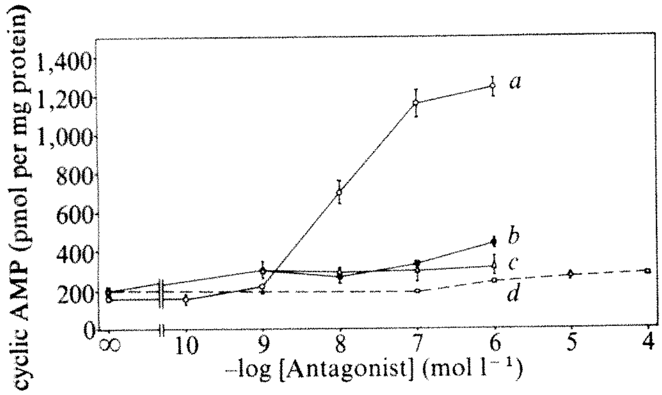

Fig. 2 Effect of cholinergic receptor blockers on the inhibition by ACh of the formation of cyclic AMP stimulated by PGE_1 in neuroblastoma × glioma hybrid cells. Besides PGE_1 all incubations contained 10 μmol l^{-1} ACh, eserine and varying concentrations of cholinergic antagonists. *a*, Obtained in an experiment separate from that which gave rise to the other curves. Cells per plate were 2.6×10^6 and 1.1×10^6, passage number 14 and 13, viabilities 91.0% and 97.7% for the cells of curves (*a*) and (*b*–*d*), respectively. Control values: *a*, eserine alone 17, ACh + eserine 10, atropine (1 μmol l^{-1}) + eserine 22, atropine + eserine + ACh 18, PGE_1 + eserine 1,100, PGE_1 + eserine + atropine 1,030; *b* and *c*, no additions or ACh alone 10, PGE_1 alone 1,200, PGE_1 + eserine 1,600. *a*, Atropine; *b*, α bungarotoxin, added 30 min before other drugs; *c*, α bungarotoxin added simultaneously with the other drugs; *d*, D-tubocurarine. Toxicity of α bungarotoxin was verified by the death of five mice injected with 4 μg of the drug.

is probably mediated by cyclic GMP. The question of whether cyclic GMP causes a reduction in adenyl cyclase activity by decreasing the number of receptor sites occupied by PGE_1, or by a more direct action on the enzyme, is under investigation.

In homogenates of a clone of mouse neuroblastoma C1300, ACh is reported to stimulate adenyl cyclase activity[17]. The effect seems to be counteracted equally well by muscarinic and nicotinic cholinergic antagonists at the high concentration of 0.1 mmol l^{-1}. It was concluded[17] that these cells contain both muscarinic and nicotinic cholinergic receptors linked to adenyl cyclase. Data showing additive effects of both types of antagonists and curves demonstrating the inhibitory effects as functions of the concentrations of the antagonists, were not given. Therefore, the above conclusion[17] seems to be premature. Our results are in agreement with findings that stimulation of muscarinic cholinergic receptors in heart[18], brain[19] and lung[20] results in elevated levels of cyclic GMP rather than cyclic AMP. Relationships between hormones elevating cellular levels of cyclic AMP and others antagonising this effect are known for other systems. Somatostatin[21], noradrenaline[22] and insulin[23-25] have been shown to counteract the increased levels of cyclic AMP in pituitary glands[21], platelets[22] and fat cells[23-25] produced by PGE_2 (ref. 21), PGE_1 (ref. 22) and adrenaline[23-25], respectively.

Our results suggest the presence of muscarinic cholinergic receptors in the hybrid cells. These cells may therefore be useful in detailed studies of the molecular properties of muscarinic receptors. Second, in hybrid cells useful as neuronal models[4] an antagonism between PGE_1 and ACh has been detected. This suggests that such an antagonism may also have an important function in the nervous system. Third, the hybrid cells may be useful model systems for studying the biochemistry of the interaction of these and other[7] hormones. Fourth, increases in cellular levels of cyclic nucleotides evoked by hormones are generally much higher for cyclic AMP than for cyclic GMP. Thus, in cells which strongly increase their level of cyclic AMP in the presence of a certain hormone, screening for hormone receptors controlling guanyl cyclase may be carried out more sensitively by measuring depressed levels of cyclic AMP than by assaying cyclic GMP. Of course, a negative result will not exclude the possibility that the hormone used stimulates formation of cyclic GMP.

This work was supported by the Sonderforschungsbereich 51 of the Deutsche Forschungsgemeinschaft. We thank Dr J. Pike for PGE_1.

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Received April 1; accepted April 30, 1975.

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Unique DNA-binding protein in the serum of patients with various neoplasms

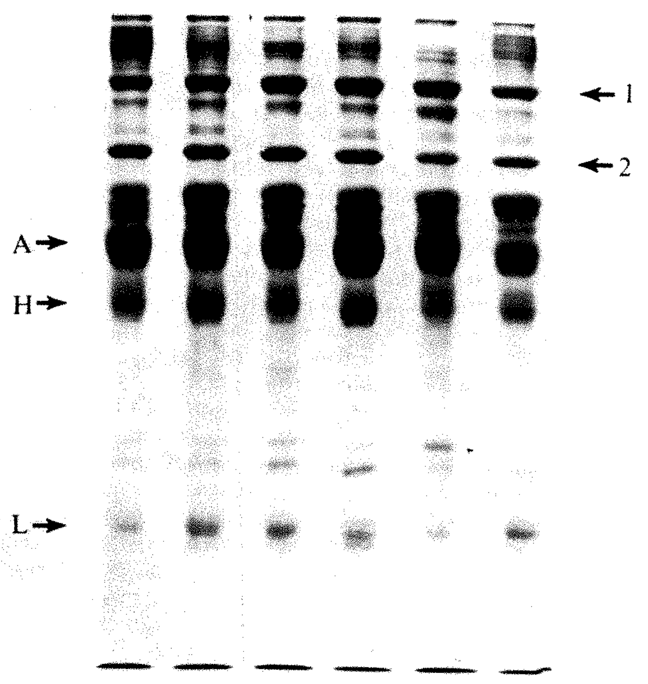
ONE of the major deficiencies in cancer medicine is the inability to detect low level malignant growth such as tumours at an early stage of development or residual malignant growth after therapy. Biochemical assays for malignancy have centred around carcinoembryonic antigen (CEA) and α-foetoprotein (AFP)[1], both of which are proteins found normally in embryonic tissues and only at low levels in mature, normal adults. Adult human serum contains a set of proteins with affinity for DNA[2] and, as we report here, foetal human serum contains additional DNA-binding proteins not observed in adult sera. Patients with a wide variety of malignant disorders have been assayed for their complement of serum DNA-binding proteins; foetal species were present in the majority of sera examined.

Human serum DNA-binding proteins were isolated by a scaled down version of our original procedure[2]. One millilitre of serum was passed over a QAE-Sephadex column to remove immunoglobulins and the eluate, after dialysis, was applied to a column of DNA-cellulose. Proteins binding to the DNA were eluted with 0.4 M NaCl. This fraction was dialysed, reduced with mercaptoethanol in sodium dodecyl sulphate (SDS), and analysed by SDS-polyacrylamide slab gel electrophoresis[3]. Figure 1 shows the Coomassie blue stained slab gel of DNA-binding proteins from six normal individuals. The bands numbered 1 and 2 are the predominant species in serum and have molecular weights of 126,000 and 94,000, respectively. The fraction at this stage is still contaminated with some albumin and immunoglobulin G. The pattern of both the major and minor protein species are invariant from individual to individual.

Foetal (cord blood) serum samples analysed in the same manner show some alterations from the pattern found in adults (Fig. 2). The most obvious change is the presence of a protein band (No. 3 in Fig. 2) of molecular weight about 36,000 with no counterpart in the adult samples. Several other changes are apparent in the minor species; a new band just below band No. 3, alterations in the pattern of bands just above albumin and between bands 1 and 2, and relatively little high molecular weight species compared with adult samples. This pattern may only approximate the actual foetal pattern since this serum was obtained from term placentae. We are proceeding under the assumption that the new species in cord blood are residual proteins from foetal development and these species may be in higher concentration in actual foetal blood.

Examination of the DNA-binding proteins in sera from cancer patients showed that foetal species were present. The most prevalent new protein in these patients was the species of foetal serum with a molecular weight of 36,000. Figure 3 shows the SDS-slab gel patterns from five individuals with various malignancies. All of these individuals exhibit a definite 36,000 species as well as other modifications in the basic adult pattern. To confirm this finding, we examined a larger number of cancer patients and scored for the presence or absence of the 36,000 species (Table 1). In all types of cancer represented, the incidence of the protein in serum is 64%. It also seems that the presence of the protein does not correlate to any particular type of malignancy, although lymphomas seem to be higher than others with a significant sample size examined. The serum samples used in this study were drawn at random from patients with and without any treatment for their malignancies. To determine if our statistics were affected by treatment, we examined the case records of 20 individuals with various carcinomas. Eleven of

Fig. 1 SDS slab gel electrophoresis of serum DNA-binding proteins from normal individuals. The samples each contained 59 μg of protein. The indicated bands are: A, Albumin; H, immunoglobulin G heavy chain; L, immunoglobulin G light chain.



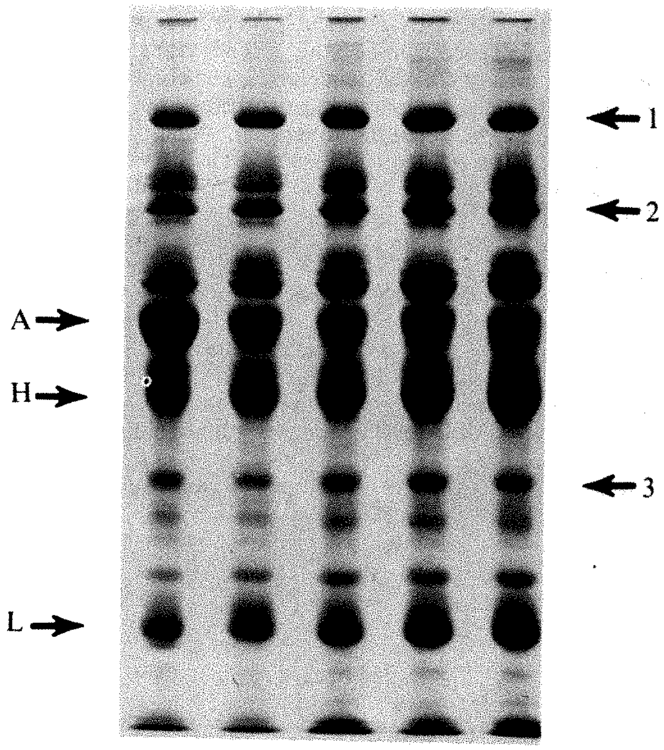


Fig. 2 SDS slab gel electrophoresis of serum DNA-binding proteins from cord blood. All samples are from individual placentae. Indications as in Fig. 1.

Table 1 Appearance of 36,000 molecular weight species in diagnosed malignancies

Serum source		No. positive / No. tested	Overall	% Positive
Normal		0/28	0/28	0
Carcinoma				
Primary site:	Breast	4/11	19/31	61
	Ovary	1/2		
	Lung	5/5		
	Prostate	0/1		
	Parotid	1/1		
	Colon	2/3		
	Bone	0/1		
	Tongue	1/1		
	Pancreas	3/3		
	Multiple site	2/3		
Leukaemia				
Type	AML	1/2	6/11	55
	ALL	1/2		
	AGL	2/3		
	CML	1/1		
	CLL	1/3		
Sarcoma				
Type	Rhabdosarcoma	1/1	1/1	100
Lymphoma				
Type	Diffuse lymphocytic	5/7	13/17	77
	Diffuse histiocytic	3/3		
	Lymphocytic and histiocytic	1/1		
	Immunoblastic	1/1		
	Poorly differentiated	2/2		
	Macroglobulinaemia	1/3		
Hodgkin's disease		3/5	3/5	60
Multiple myeloma		1/1	1/1	100
Malignant carcinoid Somatic cell type		1/1	1/1	100

the patients were positive for the 36,000 protein and nine were negative. Among the nine negative individuals at the time the serum was drawn, six were undergoing chemotherapy and in remission, two were undergoing chemotherapy but control was uncertain, and one had no treatment. Among the eleven positive individuals, three had no treatment, six were receiving chemotherapy but were not under control, and two had a history of cancer and successful therapy. These latter two patients are of particular interest since at the time the serum sample was taken no clinical manifestations of their malignancy were evident, yet the 36,000 protein was present in their serum. Both patients, one with a breast cancer and the other with a parotid carcinoma, had obvious metastasis 6 months later.

Studies on the presence of the 36,000 protein have been qualitative since no specific assay exists at this time. Purification of this species and the development of a radioimmunoassay will enable quantitative values and a larger sample size. With the results obtained so far, it seems that the range of malignancy type in which this protein appears is broader than that observed for CEA[4,5] or AFP[6]. In this respect, the presence of the protein may be of most use as a prognostic indicator and of limited use in specific diagnosis. Such a protein, however, may be of value in the early diagnosis of malignant growth.

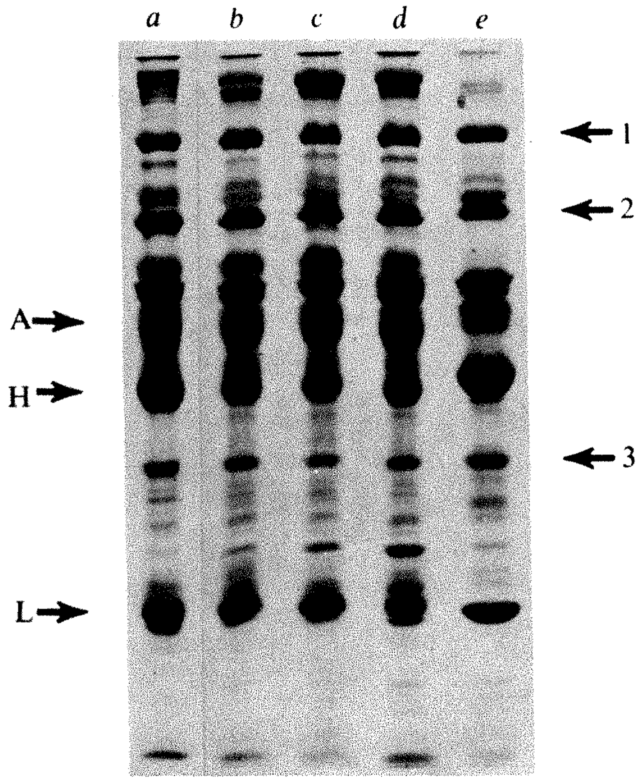


Fig. 3 SDS slab gel electrophoresis of serum DNA-binding proteins from adult individuals with various malignancies. The samples are from the sera of individuals with the following diagnoses: *a*, acute granulocytic leukaemia; *b*, histiocytic lymphoma; *c*, carcinoma of the parotid; *d*, alveolar cell carcinoma of the lung; *e*, multiple myeloma.

The function(s) that DNA-binding proteins perform in physiological fluids normally has not been determined. It has been observed, however, that alterations in the level of DNA-binding proteins in cerebrospinal fluid can be correlated with particular diseases[7] of the central nervous system. The increased level of the 36,000 species in the serum of patients with neoplastic diseases may be indicative of rapid cellular proliferation. In this respect, the protein appears as a generalised phenomenon regardless of the cell type of origin of the neoplastic disorder.

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Cross idiotypic specificity among heavy chains of macroglobulins with blood group I and i specificities

THE occurrence of cross idiotypic specificity among human red cell cold agglutinins is well known[1-3], and there is evidence that the antigen binding site is involved in these idiotypic determinants. Two distinct idiotypic systems have been described which correlate with the combining specificities of these antibodies. The first is found among IgM cold agglutinins with blood group I and i specificities and the second among proteins of IgM and IgA class with blood group Pr specificities[2]. The idiotypic determinants in each system are numerous and variable numbers are expressed on proteins from different individuals[2-4]. Sensitive binding assays showed one idiotypic determinant to be common to at least 73% of cold agglutinins with specificity for the I–i antigen complex[3]. With the antisera raised against the intact proteins the relative roles of the heavy and light chains in the cross-specificity determinants could not be ascertained because both chains in combination were required for their expression[2]. In the studies reported here, rabbit antisera against the isolated heavy chains of an anti-I and an anti-i cold agglutinin have been used and cross idiotypic specificity among the heavy chains of this group of antibodies has been demonstrated.

Cold agglutinins and macroglobulins without cold agglutinin activity were isolated and their fragments prepared as described previously[2]. The isolated heavy and light chains were dialysed against distilled water; one half of each preparation was lyophilised and the other half rendered soluble in isotonic solution by carbamylation[2]. Rabbit antisera were raised against the untreated and carbamylated heavy chains of an anti-I cold agglutinin Low[2,3], and an anti-i cold agglutinin Nic[2,3] as described previously[2]. The use of carbamylated H and L chains enabled quantitative precipitin, gel diffusion and haemagglutination tests to be carried out in isotonic solutions.

In gel precipitation tests all four antisera showed activity against determinants revealed on the isolated H chains which were not available on the intact proteins (Fig. 1). After absorption at equivalence with the heavy chains of two non-cold agglutinin macroglobulins and of human Cohn Fraction II, the antisera no longer precipitated with any of the isolated H chains, nor with the intact cold agglutinins. By passive haemagglutination (HA) assays, however, they showed specificity for cold agglutinin H chains (Table 1). The two absorbed anti-Nic H sera showed individual as well as cross specificity (HA titres of 1,024-2,048 with the homologous Nic H chains and 64-256 with Low H chains). The two absorbed anti-Low H sera, on the other hand, showed cross specificity only (HA titres of 128-512 with both Low and Nic H chains). The individual

Table 1 Haemagglutination titres of the absorbed antisera to the H chains of cold agglutinins Low and Nic

H chain coat*	Antisera against			
	Low H chains		Nic H chains	
	Untreated	Carbamylated	Untreated	Carbamylated
Cold agglutinins				
Low	512†	128	64	256
Nic	512	128	1,024	2,048
Non-cold agglutinins				
IgM Sto	<4	<4	<4	<4
IgM Cab	<4	<4	<4	<4
Cohn Fr II	<4	<4	<4	<4

The antisera were absorbed at equivalence (as determined by quantitative precipitin[2] assays) with the H chains of non-cold agglutinin macroglobulins Sto[2] and Cab[3] and of human Cohn Fraction II.

*Tanned human red cells[5] were coated with carbamylated H chains.

†Titres (reciprocals of dilutions) determined by the microtitre[6] method.

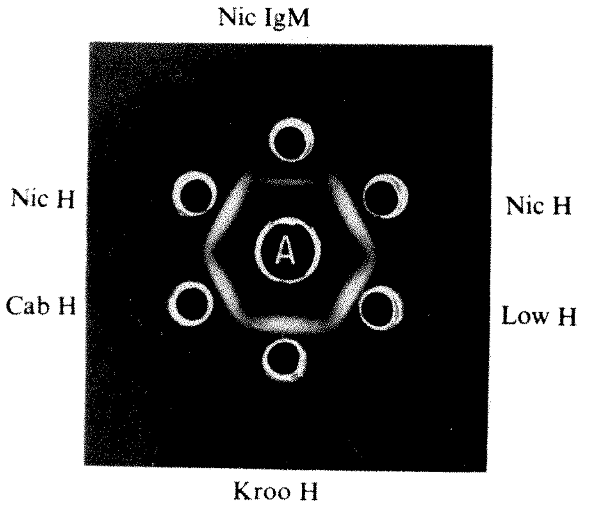
Table 2 Haemagglutination inhibition assays with absorbed antisera to H chains of cold agglutinins Nic and Low

H chain inhibitors	Antisera against					
	Nic H untreated		Nic H carbamylated		Low H untreated	Low H carbamylated
	Nic H coat (1:64)	Low H coat (1:4)	Nic H coat (1:128)	Low H coat (1:8)	Low H coat (1:32)	Low H coat (1:8)
	minimum concentration giving complete inhibition (μg ml^{-1})					
Cold agglutinins						
Nic	400	25	400	25	6	50
Low	>1,015	127	>1,015	254	32	127
Kroo	>1,035	129	>1,035	65	32	32
Non-cold agglutinins						
IgM Sto	>965	>965	>965	>965	>965	>965
IgM Cab	>836	>836	>836	>836	>836	>836
Cohn Fr II	>1,085	>1,085	>1,085	>1,085	>1,085	>1,085

The figures in parentheses indicate the antiserum dilutions used with the various H chain coats.

idiotypic specificity of the two anti-Nic H sera was confirmed in inhibition of HA assays where the homologous H chain (Nic) was used as the red cell coat (Table 2): the H chains of Nic were inhibitory while those of cold agglutinins Low and Kroo[3] were not inhibitory at the concentrations tested. On the other hand, when a heterologous cold agglutinin H chain (Low) was used as the protein coat, the two anti-Nic sera clearly showed cross idiotypic specificity among the H chains of cold agglutinins Nic, Low and Kroo. The anti-Low H sera again showed cross idiotypic specificity only: all three cold agglutinin H chains could inhibit the haemagglutination of the homologous H chain coat. The specificities of the four antisera, whether raised against the untreated or the carbamylated H chains, were satisfactorily demonstrated using carbamylated H chains as antigens.

Fig. 1 Gel precipitation reactions of an unabsorbed antiserum (A) raised against the untreated H chains of cold agglutinin Nic. The peripheral wells contained native cold agglutinin Nic (1 mg ml^{-1}), carbamylated H chains of cold agglutinins Nic, Low, Kroo[3] and of the non-cold agglutinin macroglobulin Cab[3] (0.2 mg ml^{-1}). The Nic H precipitin line spurs over that formed by intact IgM Nic indicating that the antiserum has activity toward additional determinants present on the isolated H chains. Similar reactions were obtained with the other three antisera raised against the untreated or the carbamylated cold agglutinin H chains.



Further studies were carried out to confirm the specificity of the absorbed antisera for dissociated cold agglutinin H chains (Table 3). Isolated L chains of the three cold agglutinins were not inhibitory at the concentrations tested; nor were whole cold agglutinin molecules or fragments with intact HL chain bonds; however, after reduction of whole cold agglutinins and chain dissociation by the method of McLaughlin and Solomon[7], inhibitory activity was revealed. The HA inhibition studies were therefore extended to include five additional anti-I–i cold agglutinins and four non-cold agglutinin macroglobulins after reduction, alkylation and chain dissociation. With the exception of anti-I Ma the cold agglutinins tested by this method showed inhibitory activity and could be clearly distinguished from the non-cold agglutinin proteins (Table 3). These findings are in agreement with previous studies[2,3] using idiotypic antisera against intact proteins which showed that these cold agglutinins, with the exception of Ma, had strong idiotypic cross reactions; anti-I Ma which differs markedly from the other cold agglutinins in its antigen binding specificity[8,9] was shown to be deficient in some of the idiotypic determinants associated with these proteins.

The idiotypic determinants recognised by the anti-H chain antisera seem to be different from those recognised by antisera

Table 3 Haemagglutination inhibition assays with an absorbed antiserum to untreated H chains of cold agglutinin Low showing cross idiotypic specificity for reduced-dissociated H chains (red cell coat–Low H chains; antiserum dilution 1:32)

Inhibitors	Minimum concentration giving complete inhibition (μg ml^{-1})
Cold agglutinins	
Low H chains	20
Low L chains	> 1,000
Low Fab	> 1,230
Low F(ab′)$_2$	> 1,238
Low IgM reduced-dissociated*	18
Nic† H chains	24
Nic L chains	> 1,000
Nic IgM	> 1,000
Nic Fab	> 1,280
Nic F(ab′)$_2$	> 2,840
Nic IgM reduced-dissociated	60
Step IgM reduced-dissociated	17
Da IgM reduced-dissociated	950
Sch‡ IgM reduced-dissociated	500
Den†‡ IgM reduced-dissociated	140
Ma IgM reduced-dissociated	> 1,750
Non-cold agglutinins	
Mi IgM reduced-dissociated	> 7,800
Gr IgM reduced-dissociated	> 5,000
Dav‡ IgM reduced-dissociated	> 8,850
Ham‡ IgM reduced-dissociated	> 8,000
Cohn Fr II reduced-dissociated	> 5,000

*HL dissociation without chain separation was carried out by reduction, alkylation, dialysis against 1 M acetic acid followed by dialysis against distilled water, as previously diescribed[7].
†Anti-i cold agglutinin; all others had anti-I specificity.
‡IgM λ proteins; all others were IgM κ.

raised against the intact cold agglutinins. The latter fail to react with dissociated H and L chains[2]. The findings with the anti-H chain sera strongly suggest that the H chains of this group of antibodies with related antigen bindings specificities have similarities in their hypervariable regions—the absorption procedures and the negative findings with the H chains of pooled human IgG would make V_H subgroup specificities[10] in the absorbed antisera unlikely.

Similar studies now under way with the light chains of these antibodies may provides additional important information regarding the selection of hypervariable regions in these antibodies, some of which have selected κ chain subgroups and others λ chains[2,3,12].

In highly inbred strains of mice the H and L chains of myeloma proteins with the same antibody activity and the same idiotypic determinants usually have identical or very similar V regions, that is, identical V_L and V_H subgroups (framework regions) as well as identical or very similar hypervariable regions[13,21] (site determining regions). In partially inbred rabbits, however, occasional proteins with differing V region subgroups or allotypes have been reported which show cross-idiotypic specificity[14-16]; these have provided support for the insertional model[17] for the coding of the site determining regions of antibodies. This model proposes that separate genes code for the site-determining and the framework portions of the V region. The cold agglutinins and certain other antibodies with restricted specificities[18-20] in unrelated persons offer an excellent opportunity for studying the selection of site determining regions in relation to other V region structures on H and L chains. The availability of discriminating idiotypic antisera against isolated chains should prove useful in the screening and selection of H and L chains and their fragments for variable region sequencing.

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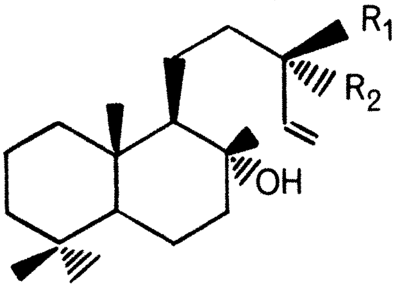
Errata

In the article "Control of rust diseases by diterpenes from *Nicotiana glutinosa*" by J. A. Bailey, G. A. Carter, R. S. Burden and R. L. Wain (*Nature*, **255**, 328; 1975) Fig. 1 was incorrect. The correct form is reprinted below.



In the article "Observations of a nonlinear interaction involving three electromagnetic waves in a laboratory magnetoplasma" by P. J. Christiansen, M. J. Giles, G. Martelli and N. D. Wells (*Nature*, **254**, 685; 1975) there was in error in Fig 3. The correct figure and legend are reprinted below.

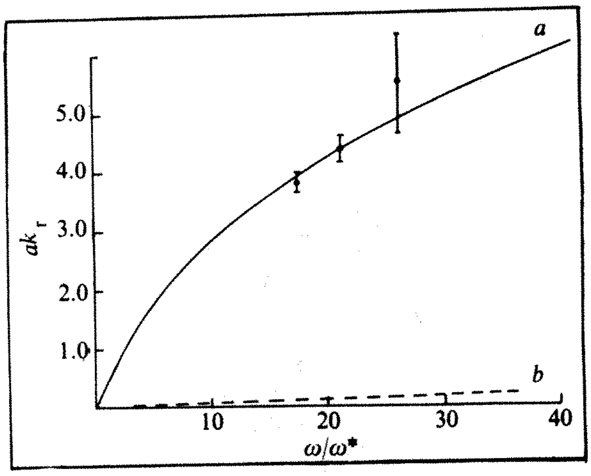


Fig. 3 Experimental data fitted to the theoretical dispersion curve (*a*) of low frequency whistlers (helicon waves[6]). In the axes labels *a* is the tube radius, k_r is the real part of the wavenumber and $\omega^* = B_0/4\pi nea^2$. The inferred value of the plasma frequency, *n* ($\sim 1.2 \times 10^{12}$ cm^{-3}) is in good agreement with independent Langmuir probe measurements[1]. *b*, Dispersion curve for quasistatic waves.

reviews

Weighted clocks and steam engines

Eric H. Robinson

The Maze of Ingenuity: Ideas and Idealism in the Development of Technology. By Arnold Pacey. Pp. 350. (Allen Lane: London, January 1975.) £5.50.

HERE is a book that should immediately be put into paperback and placed in the hands of undergraduates everywhere and in all disciplines. Its subject matter ranges from the cathedral builders of the Middle Ages to the prophets of systems analysis in our own century. Yet Dr Pacey is nowhere superficial. His discussion of "A century of invention: 1250–1350" includes, for example, an exciting examination of the thesis that the weight-driven clock was not the product of monastic needs but was inspired by the imaginative desire to parallel the movements of the planets in a model here below. The author constantly stresses non-economic motivation in the development of technology and, in this respect, seems to share a point of view with that great historian of metallurgy, C. S. Smith, whose work he never cites. It is very refreshing, moreover, to see a history of technology that refers truly to the technology of Asia (China, India and Japan) as well as that of the West, and moves with confidence in discussing the Islamic contributions to mediaeval and renaissance technology. One chapter is entitled "The effects of mechanisation in Europe and Asia: trends in technology between 1810 and 1870".

Though Dr Pacey is best known for papers on various aspects of the Industrial Revolution in Britain, it is in his discussion of mediaeval and renaissance architecture that he excels in this book. Not that he does not have illuminating comments on John Smeaton, James Watt, Peter Ewart, William Strutt and other well known figures of the late eighteenth and early nineteenth centuries; but his discussion flows most freely when he is not too closely involved with biographical detail. The book seems to be more concerned with broad imaginative ideals within different cultures and with the state of craftsmanship in different civilisations than it is with the heroic inventor, yet the biographical approach has a strong hold upon the author and sometimes seems to run away with him. On occasion, indeed, it seems to lead to self-contradiction. For example, on page 226, Dr Pacey quotes from a letter by George Lee to illustrate "the abysmal ignorance of algebra and the calculus which prevailed among even well-read engineers in Britain at this time" but on page 240 quotes with approval Robert Owen's judgment of Lee as "one of the most scientific men of his day, who was considered a man of very superior attainments, having been highly educated, and being a finished mathematician".

I would question in particular Dr Pacey's evaluation of some eminent technologists, but found the book constantly illuminating, soundly researched, ranging widely over different disciplines and always civilised in tone. How many historians of technology could be expected to comment with insight upon the poetry of Alexander Pope? Dr Pacey, however, is wrong in drawing a contrast between Britain's economically-motivated uses of the steam engine for mills and the Russian or French use of it to water gardens. One of the first uses of the Boulton and Watt steam engine in Britain was to water a landed gentleman's garden, and if you are pumping water you may as well use it to refresh your carnations or your cabbages as to drive a loom or power a brewery. In fact some of Dr Pacey's differentiations between technology motivated by ideals and technology motivated by gain are a little too simplistic but this is the best book on the history of technology over a wide period to appear for a long time.

Amniotic fluid

R. W. E. Watts

Amniotic Fluid: Physiology, Biochemistry and Clinical Chemistry. (Current Topics in Clinical Chemistry, vol. 1.) Edited by Samuel Natelson, Antonio Scommegna and Morton B. Epstein. Pp. xv+386. (Wiley: New York and London, 1974.) £10.80.

THIS book results from an Interdisciplinary conference on amniotic fluid held in May 1973, under the auspices of the American Association of Clinical Chemists, and the Chicago Gynaecological, Immunological and Paediatric Societies. Each session is represented by a section of the book.

Section 1 includes papers on the morphology and development of the amnion, and the dynamics and chemistry of the amniotic fluid. The latter is concerned mainly with a consideration of the commoner metabolites although there is a report of recent work on measurements of adrenalin and noradrenalin.

Section 2 includes papers on immunoglobulins and other protein constituents of amniotic fluid, electrophoretic and immunological analysis of amniotic fluid, placental lactogen, disseminated intravascular coagulation in obstetrics, and papers dealing with problems arising from Rhesus incompatibility. Section 3 deals with the obstetrical problems such as the technique of amniocentesis, use of ultrasonography, the value of lecithin, sphingomyelin and oestrogen measurements in clinical practice.

The last section deals with the very rapidly growing field of the diagnosis of genetic disorders, and there have been new developments even in the small range of topics covered since the conference was held. Newton Ressler's contribution discusses a pitfall which must be avoided in this work and it should be read carefully by anyone entering the field. Abraham Saifer and his colleagues describe their extensive experience of mass screening for heterozygous carriers of Tay Sachs disease, and in the prenatal diagnosis of this disorder. This chapter is of particular interest as a pointer to what can be achieved when necessary in this area of preventive medicine.

The book contains much of interest to biochemists and clinicians, but it is patchy and uneven in coverage and in style. Some of the contributions would have profited from more severe editing so that they less obviously resembled a transcript of the spoken contribution. Regrettably, a few of the papers contain mainly material which is already well known. It is, however, a useful book for the specialist and for the library. □

Pure Che[illegible]stry Library.

Structure and role of plasma proteins

Structure and Function of Plasma Proteins, vol. 1. Edited by A. C. Allison. Pp. viii+316. (Plenum: London and New York, 1974.) $28.90.

As the title indicates, this book is concerned with both the structure and the function of plasma proteins. Unfortunately, with the exception of the immunoglobulins and albumin, available structural data are still mainly of a physiochemical character but even so information of this kind is well presented for a number of plasma proteins. Most, though not all, selected for inclusion have at least one known function. It is evident that in several cases these functions and their normal physiological roles are identical, though in other cases true physiological roles are still uncertain; the existence of analbuminaemics who often enjoy excellent health must serve to underline this point. In this regard it is disappointing that the chapter on albumin deals only with "aspects of chemical behaviour and structure" and that, in consequence, no comment is made on the function of albumin. It is indeed curious that a book with such a title does not as yet include any information concerning the function of that protein which is present at the highest concentration in plasma. But at least the information that is given about albumin is excellently presented and includes an interesting discussion of the macro and microheterogeneity of this protein. The opposing views of two schools of thought on the complex matter of the p*H* dependent N–F transition of albumin are very fairly presented.

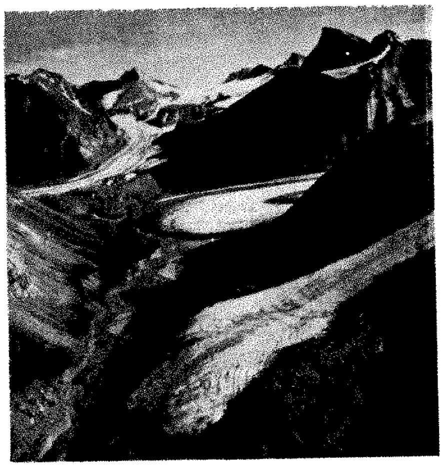
Very satisfactory descriptions are given of present knowledge concerning both haptoglobin and haemopexin, two proteins usually considered to be of known function. As with albumin, genetic deficiency (or at least very low levels of haptoglobin) is compatible with reasonable health and it certainly is "important to know if complete absence of haptoglobin is compatible with life".

An especially warm welcome must be given to the chapter on acute phase reactants. As I have mentioned, the physiological roles of certain of these proteins, such as fibrinogen, are clear whereas the stimuli which bring about increased synthesis after injury of this and other acute phase proteins are little understood. Although on the basis of experiments using the isolated perfused rat liver it has been suggested that hormonal factors are of prime importance, evidence obtained by perfusion of physiologically normal livers suggests otherwise; a very rapid rate of synthesis of haemopexin has been obtained when such livers are perfused (p. 42). Since haemopexin is not an acute phase reactant the finding that in the presence of hormones perfused livers from normal rats synthesise acute phase reactants at very high rates falls into a new perspective. Evidently, the simplest hypothesis at this time is that perfused livers synthesise many proteins (albumin is a major exception) at a greatly increased rate. Such behaviour may most reasonably be ascribed to the presence *in vivo* of inhibitors which cease to function under perfusion conditions.

In spite of the fact that certain chapters are out of date the book represents a valuable contribution to the field.

A. H. Gordon

Life and science in cold environments



Glaciated arctic landscape on the north-eastern coast of Baffin Island.

Arctic and Alpine Environments. Edited by Jack D. Ives and Roger G. Barry. Pp. xviii+999+47 plates. (Methuen: London, November 1974.) £35.00.

In recent years Man and his technology have begun to invade the cold arctic regions for science, minerals and simply for amenity. So this book, dedicated to Professor Carl Troll in acknowledgment of his extensive work on altitude geo-ecology, is a very timely synthesis of arctic and alpine work. It is edited by the director and coworker of the Institute of Arctic and Alpine Research at the University of Colorado and is largely written by colleagues working there or at government departments or universities in the USA or Canada; two authors are German, one Norwegian and one a New Zealander. Each contributor is an authority in his field and is very readable and generally succinct; some of the chapters are scholarly masterpieces of condensation.

The awful price of £35.00 will deter most private purchasers but it is only the absence of exploration sagas and of contributions concerning social sciences and economic aspects that prevents the book from qualifying as an encyclopaedia. The 1,000 pages, which include a glossary, an index and 49 black and white plates inconveniently located at the end, embrace 37 papers, each of which has an extensive bibliography. They are divided into sections covering present and past environments, present biota and its development, abiotic processes, man in cold environments and man's impact on the environment. A more practicable approach would have been to present a format in three parts covering physical, biological and human (including technological) aspects. Expensive photographs could have been replaced with sketches where possible, and the number of diagrams could have been increased, even though 200 are already included.

Some of the papers treat elements of the environment in a systematic manner: the paper entitled "Snow" is an erudite summary of this ubiquitous characteristic of both arctic and alpine environment, but it says nothing of drift snow and would benefit from more diagrams. On the other hand regional themes dominate other papers, such as those on "Arctic Hydrology" and "The Present Ice Cover". Many of the papers, however, combine both the systematic and the regional approach—that on "Radioecology", for example—but very few treat 'arctic' and 'alpine' areas as similar regions; indeed, one of the achievements of the book is to pinpoint the differences between them. One of the longest and most fascinating papers explains how the alpine and artic tundra are combined in a single tundra biome, an approach which, though having some justification where plants are concerned, has far less in the case of animals, particularly vertebrates, as not a single mammal is common to both arctic and alpine tundra. Controlling the differences between the two environments are length of day, solar radiation patterns, topography and moisture. Distances involved are so much greater in the arctic and it encloses an ocean which (even though largely ice covered) provides a further contrast.

Extremely brief conclusions from many atmospheric models and palaeotemperature profiles form only a small part of a brilliant precis on palaeoclimatology. Radiation over all the different time scales is a dominant variable in the many heat balance expressions used through the book. Better coordination of these would be a blessing to the student reader. And more data from The International Biological Programme, from the Alaska pipeline and from many smaller projects is now available to the rapidly expanding, corporate body of knowledge so lucidly defined in this most creditable collection.

H. Lister

Introduction to Biophysical Plant Physiology. By P. S. Nobel. Pp. xii+488 (W. H. Freeman: San Francisco and Reading, 1974.) £7.10.

THIS excellent book has been written essentially for advanced undergraduate and postgraduate study and is an extension of an earlier publication by the same author (*Plant Cell Physiology: A Physiochemical Approach:* Freeman, 1970). Its aim is to show how basic physical and chemical principles can be applied to whole plant and plant cell physiology.

The first three chapters are extended and improved versions of the corresponding chapters in the original book and give thorough descriptions of the quantitative and experimental aspects of water and ion distribution and movements in plant cells. These phenomena are discussed in terms of both classical and irreversible thermodynamics. As in the earlier edition, Chapter four gives an outline of the properties of light and its absorption by molecules and serves as an introduction to chapters five and six. These deal with the light reactions of photosynthesis and the main features of energy conversion in the chloroplast and mitochondrion.

The remaining chapters are additions to the original book and cover various aspects of leaf and whole plant interaction with the environment, concentrating particularly on water, gaseous and energy fluxes within and at the surfaces of the plant.

The book contains a number of numerical examples for the reader to test his understanding of the concepts presented.

The text itself is fairly readable, although to some extent there is inconsistency in the degree of explanation of some of the concepts presented. Overall, I would like to congratulate the author for producing a unique and valuable text-book. It is reasonably priced and I thoroughly recommend it to all those interested in a quantitative approach to plant physiology—both at the teaching and research level—who wish to place this aspect of plant biology on a firmer physical and chemical basis. **J. Barber**

The Vanishing Lichens: Their History, Biology and Importance. By D. H. S. Richardson. Pp. 231. (David and Charles: Newton Abbot, London and Vaucouver, 1975.) £5.25.

THE title of this book is unfortunately very apt. No other major group of plants has suffered so much from the effects of atmospheric pollution. The area of distribution of many species has contracted dramatically during the past century and all but the few pollution-tolerant species really are vanishing—and not all that slowly.

But this is not a book about the effects of pollution on lichens. Rather, it gives a readable and enjoyable account of the group as a whole; because it is deliberately aimed at a popular audience, there is a necessarily heavy emphasis upon the importance of lichens to man and how he uses them.

The book is assuredly successful in achieving its objective. Furthermore, there is much even to intrigue specialists such as myself. Would that more scientists took time off to write books which are genuinely entertaining yet keep within the bounds of scientific accuracy. In the long run it will be books like this which will help the cause of conservation amongst the public at large, rather than patronising diatribes in the Sunday colour supplements. People need to develop affection for wild life, not just a dutiful respect.

There is a slight air of zaniness—characteristic of quite a number of other books published by David and Charles; one can't help feeling that James Thurber selected some of the line drawings. It is a pity, though, that the need to save money has forced the publishers to use poor or just bad reproduction of plates which were very good in the original. The picture of lichens colonising power-station cooling-towers on page 7 means little.

Plant life

Lichenologists at work; from Richardson's book.

Curiously, the farther the author gets away from explaining science, the better he is. Particularly fascinating are the many little side-trips away from lichens, that occur in the narrative. Thus, a description of the use of lichens for dating Easter Island megaliths goes on, out of sheer interest, to say something about megaliths in their own right.

Dr Richardson is to be congratulated on writing the most entertaining book on lichens to date. **D. C. Smith**

Crop Physiology: Some Case Histories. Edited by L. T. Evans. (Cambridge University Press: London, February 1975.) £8.00; $23.50.

IN a world in which a depression in the crop yield of one major food-producing country can have international repercussions or even result in mass starvation, books which critically evaluate important aspects of current knowledge of agricultural crops are to be welcomed.

In this book, authoritative contributors from Australia, the USA and Japan examine the physiological basis of yield in nine important crops: maize, sugar cane, rice, wheat, soybeans, pea, potato, sugar beet and cotton. The introductory chapter deals with some more general aspects of world food supply, crop evolution and the origins of crop physiology. In the final chapter Dr Evans discusses and collates the information obtained about individual crops and considers possible applications to agronomic techniques and plant breeding. Each chapter is followed by an extensive list of references.

The crops chosen illustrate the diversity of 'yield' in the agricultural sense; the tubers of the potato, the sucrose content of the sugar cane stem, grains of the cereals, and fibres of the cotton boll are all considered. The physiological pathways and limitations to attaining maximum yields are equally diverse and the separate chapters discuss these topics from rather contrasting viewpoints, ranging from the largely agronomic (maize) to the more theoretical approach of crop modelling (sugar beet). Most chapters are not confined to purely physiological aspects and include useful sections on origins, evolution, adaptability and distribution of the crop and on many aspects of growth from sowing to harvest. There is a strong awareness throughout the book of the potential applications to plant breeding arising from current knowledge of crop physiology. This is emphasised by Dr Evans who stresses the value of the production of new ideotypes, discusses the bases of yield assessment—he considers that the efficiencies of water use and of phosphate use will become more important in the future—and briefly examines the efficiency of energy use in agriculture.

The book is extremely readable and well presented and can be recommended not only to crop physiologists but also to agronomists and plant breeders. **David Wilson**

announcements

International meetings

August 3–9, **Nutrition,** Kyoto, Japan (Professor H. Mitsuda, Research Institute for Production Development, Morimoto, Shimagamo, Sakyoku, Kyoto 606, Japan *or* Kyoto International Conference Hall, Takara-ike, Sakyoku, Kyoto 606, Japan).

August 4–7, **Neutron activation analysis,** Cambridge (Mr C. H. Gill, Conference Secretary, Neutron Division, Marconi Elliott Avionic Systems Ltd, Elstree Way, Borehamwood, Herts WD6 7RX, UK *or* Dr J. Pauwels, Euristop Office, Commission of the European Communities, 200 rue de la Loi, B1040 Brussels, Belgium).

August 4–7, **Image processing for 2D and 3D reconstruction from projections,** California (Optical Society of America, 2000L Street, NW, Washington, DC 20036.

August 4–7, **Odonatology,** Lancaster (Dr T. T. Macan, Freshwater Biological Association, Ferry House, Far Sawrey, Ambleside, Cumbria, UK).

August 4–9, **Biophysics,** Copenhagen (Fifth International Biophysics Congress, c/o DIS Congress Service, 3 Knabrostaede, DK 1210 Copenhagen K, Denmark).

August 5–6, **Neutron diffraction,** Petten, Netherlands (Neutron Diffraction Conference, Reactor Centrum Nederland, Petten (NH), The Netherlands).

August 6–8, **Applications of X-ray analysis,** Denver, Colorado (Dr C. O. Ruud, Metallurgy and Materials Science Division, Denver Research Institute, University of Denver, Denver, Colorado 80210).

August 7–14, **Crystallography,** Amsterdam (J. N. King, Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, UK *or* Organisatie Bureau Amsterdam Bv, PO Box 7205, Amsterdam, The Netherlands).

August 11–15, **Astronomy,** Leicester, UK (Professor K. A. Pounds, Chairman, Local Organising Committtee, Department of Physics, University Road, Leicester LE1 7RH, UK).

Person to Person

Somantin. Researcher concerned about possible implications of theories concerning somantin and cataglykin (fragments of growth hormone which may be important in control of blood glucose concentration, and development of some cases of diabetes), wishes to know results of relevant studies, besides those of Bornstein: *Nature,* **217,** 707 (1968) or Bornstein, J., *et al., Postgrad. med. J.,* **49** (suppl.), 219–242 (1973), (Dr P. L. Schwartz, Department of Clinical Biochemistry, University of Otago Medical School, PO Box 913, Dunedin, New Zealand).

Biochemical journal. Volumes 62–105 (1956–67), complete, unbound, will be presented (delivered) to research laboratory or educational institution 'with a good cause'. Applicants should be prepared to bind volumes and indicate source of presentation. Apply by June 30 to: Dr R. Bernstein, SA Institute for Medical Research, PO Box 1038, Johannesburg, South Africa.

Leeds house. Academic going on sabbatical seeks family for house in Leeds. From August, 1975–September, 1976. Possible to commute to Bradford or York. Four bedrooms, large kitchen and reception rooms, cellars (for hobbies or storage), large garden, garage (Dr S. Baumberg, Department of Genetics, University of Leeds, Leeds LS2 9JT, Yorkshire, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robbie Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

University of Oxford. Eleventh Annual Report of the Delegates of the Science Area for the year ending 31 July 1974. (Supplement No. 1, to the *University Gazette,* Vol. cv, March 1975.) Pp. 139. (Oxford: The University, 1975.) £1.25. [14

Greenwich Observatory: 300 Years of Astronomy. Edited by Colin A. Ronan. (An Exhibition at the National Maritime Museum telling the story of Britain's oldest scientific Institution, the Royal Observatory, at Greenwich and Herstmonceux.) Pp. 63+16 coloured plates. (London: Times Books, 1975.) [24

The effect of Man on the Landscape: the Highland Zone. Edited by J. G. Evans, Susan Limbrey and Henry Cleere. (Research Report No. 11.) Pp. vii+129. (London: The Council for British Archaeology, 1975.) £7.50 [34

University of Oxford. Annual Reports, 1972-4. Pp. 20. (Oxford: The University, 1975.) £1. [44

Science Research Council. List of Research Grants Current on 1 October 1974. Pp. 182. (London: Science Research Council, 1975.) [74

Daresbury 1974—Progress Report. Pp. 52. (Daresbury, Warrington: Science Research Council, Daresbury Laboratory, 1975.) [74

The Zoological Society of London. Annual Report 1974. Pp. 48. (London: The Zoological Society of London, 1975.) [104

Flora of Tropical East Africa. Edited by R. M. Polhill. Cannabaceae. By B. Verdcourt. Pp. 3. 15p. Cochlospermaceae. By B. Verdcourt. Pp. 3. 15p. (London: Crown Agents for Oversea Governments and Administrations, 1975.) [104

The United States Binary Nerve-Gas Programme: National and International Implications. By Julian Perry Robinson. (ISIO Monographs, First Series, No. 10.) Pp. 51. (Brighton: Institute for the Study of International Organization, University of Sussex, 1975.) £1.50; $4. [104

Maincrop Potato Production, 1973. Pp. 47. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) 25p. [104

The Macaulay Institute for Soil Research. 1973/1974 Annual Report. (No. 44). Pp. 95. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1975.) [104

Natural Environment Research Council. National Institute of Oceanography, Collected Reprints, Vol. 21. Reprints 886-952. (Wormley, Godalming: National Institute of Oceanography, 1973.) [104

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1280: An Application of Normal Mode Theory to the Retrieval of Structural Parameters and Source Mechanisms from Seismic Spectra. By F. Gilbert and A. M. Dziewonski. Pp.187-269. UK £3.50; Overseas £3.60. Vol. 278, No. 1281: Field Emission and Field Ionization in Liquid ^{4}He. By A. Phillips and P. V. E. McClintock. Pp.271-310. UK £1.70; Overseas £1.75. (London: The Royal Society, 1975.) [114

Fundamental Research in an Industrial World. By Professor Alastair Cameron. (Inaugural Lecture, 22 January 1974.) Pp.199-210. (London: Imperial College of Science and Technology, University of London, 1975.) 60p. [114

Other Countries

Annual Report of 1972, 1973, of the Keikyu Aburatsubo Marine Park Aquarium, No. 5, 6. Pp.47. (Miura City, Kanagawa, Japan: The Keikyu Aburatsubo Marine Park Aquarium, 1975.) [44

New Theory of Faster-than-Light Relativity (Classical and Quantum) with Wonders of Implied Results for Public Interest and Understanding. By Dr. B. B. P. Sinha. Pp.92. (Guelph, Ontario: Institute of Science and Mathematics, PO Box 1574, 1974.) [44

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 237: Carboniferous Ammonoids and Stratigraphy in the Canadian Arctic Archipelago. By W. W. Nassichuk. Pp.240 (18 plates). $7. Memoir 376: Geology of the Antigonish Highlands, Nova Scotia. By D. G. Benson. Pp.92. $4. Memoir 377: Geology of Kognak River Area, District of Keewatin, Northwest Territories. By K. E. Eade. Pp.66 (13 plates). $4. (Ottawa: Information Canada, 1974 and 1975.) [44

Geological Survey of Western Australia, Annual Report for 1973. Pp.109. Bulletin 123: The Geology of the Blackstone Region, Western Australia. By Dr. J. L. Daniels. Pp.257. (Perth: Geological Survey of Western Australia, 1974.) [44

Republic of the Sudan. Ministry of Agriculture—Agricultural Research Division. 1967-1968 Annual Report of the Gezira Research Station and Substations. Pp.vi+273. (Wad Medani: Gezira Research Station, 1975.) [74

Report of the Council for Tobacco Research—U.S.A., Inc., for 1974. Pp.89. (New York: The Council for Tobacco Research U.S.A., Inc., 110 East 59th Street, 1975.) [74

Stockholm International Peace Research Institute. Report of Activities 1974. Pp.27. Nuclear Disarmament or Nuclear War? Pp.27. Preventing Nuclear-Weapon Proliferation: An Approach to the Non-Proliferation Treaty Review Conference. Pp.37. (Stockholm: Stockholm International Peace Research Institute, 1975.) [74

Suomen Geodeettisen Laitoksen Tiedonantoja/Reports of the Finnish Geodetic Institute. 75-1: Program for the Adjustment of the Finnish First Order Triangulation. By Jorma Korhonen. Pp.11. 74-5: The Laser Rod Comparator. By Mikko Tekalo. Pp.14. (Helsinki: Suomen Geodeettisen Laitoksen Tiedonantoja, 1974 and 1975.). [84

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Entomology, 1973/1974. Pp.115. (Canberra: CSIRO, 1975.) [94

Australian Journal of Zoology, Supplementary Series. No. 33: Studies on Australian Muscidae (Diptera). VI. New Species and Records of *Dichaetomyia* Malloch, *Hennigiola* Pont and *Prohardyia* Pont. By A. C. Pont. Pp. 30. No. 34: A Revision of World Acanthosomatidae (Heteroptera: Pentatomidae)—Keys to and Descriptions of Subfamilies, Tribes and Genera, with Designation of Types. By R. Kumar. Pp. 60. (East Melbourne: Editorial and Publication Service, CSIRO, 372 Albert Street, 1974.) [94

nature

June 26, 1975

A hundred million dollars' worth of holes

LAST year, at the instigation of Dr Eugene Shoemaker of the California Institute of Technology, about 50 United States geologists, geophysicists and representatives of the drilling industry put their heads together to talk about deep drilling in the Earth's crust for scientific purposes. Not surprisingly, they came out in favour of it. Their proposal, aimed at anyone who will listen, is for a large scale continental drilling programme within the United States and is just published*. The recommendations are sufficiently wide-ranging and expensive (indeed they have rather a 1960s air to them) to merit a long critical look by the scientific community and some very careful thinking in Washington.

The primary purpose of the Continental Drilling Project (CDP), according to the report, is "to provide a sound scientific base that will aid government in establishing national energy policy and that will assist industry in the discovery and development of new energy policies". Then, a page later, the "objective of the proposed drilling program is not for mineral exploration itself but for scientific data ... helpful to geoscientists in developing sound theories on mineral deposit locations". But then a quarter of the funds are to go to investigations of the nature of faulting and earthquakes—the public safety line. All this in a frantic ten pages of foreword, preface, summary and recommendations and introduction. Clearly the net is being cast wide for potential sponsors.

Holes from 1 to 10 km deep could, it is claimed, make major contributions in at least the following fields:

- Lateral penetration of a fault zone to study near-fault conditions, even with the idea of thinking seriously of earthquake control.
- Drilling into hydrothermal systems and active magma chambers in an attempt to understand their physics and geology.
- Determination of the thermal structure of the Earth's crust.
- Investigation of the state of ambient stress in the crust.
- General studies on the deep geology of the United States.

In addition, a programme of shallow drilling (down to 300 m) is proposed in which three teams sweep over the United States, drilling 500 holes a year for a variety of purposes. This last programme, at $1 million a year, looks sufficiently sensible (and certainly necessary) to be implemented independently of the success or failure of the rest of the proposals.

What of the remainder—can it be done, should it be done and if so, by whom?

The report makes it clear that drilling to a depth of 10 km in crystalline rock will be pushing technology quite hard. Holes to that depth exist in sedimentary formations (in pursuit of oil), cost up to $10 million and take up to 700 days to drill. In hard rock the costs would be comparable and the time required somewhat greater, but costs and times would obviously increase if extensive scientific investigations were carried out simultaneously. The instrumentation requirements for such work demand considerable improvements in temperature protection over present-day equipment But there do not seem, as far as the technology goes, to be any fundamental obstacles.

Should it be done? The past twenty years have seen some chequered fortunes in major earth-science ventures. (The name of Mohole inevitably springs to mind, but so also does the Joides programme and its successor, the Deep Sea Drilling Project. The report makes only very passing mention of the latter, and none at all, of course, of Mohole.) What emerges clearly is that not all the proposals discussed have equal merit and that it would perhaps be a mistake to try to sell a $100 million package on the basis that everything fits together. For instance, drilling laterally into the San Andreas Fault might be worth the investment of $10 million or $20 million (but it is surely imprudent to talk yet of control), whereas many of the less specific projects aimed at generalities like understanding the underlying structure of the North American continent barely warrant the same attention. It is particularly unfortunate that the whole is tied together with vague claims about energy policy and mineral resources. The veneer which it is necessary to apply to proposals these days is often distinctly thin, if not transparent. A wise decision in Washington would be to be very selective of the pack and to ignore the packaging.

And if support is forthcoming, who will get it? Drilling companies will, of course, and will handily find the government supporting their research and development. On the scientific management front, the division has somehow to be worked out between government institutions, particularly the Geological Survey, and the universities. Giving the lion's share to the Geological Survey will cause a lot of bitterness in the academic world among those who think the survey writes its own cheques, but at least the job will get done. What the survey fears, no doubt, is that it will be entrusted with day-to-day running, with the universities coming in when things get exciting and creaming off all the interesting material.

Finally it is regrettable that in the earth sciences, traditionally most internationally minded, it is now desirable to write a report which barely touches on international collaboration. A paragraph is devoted to mention of collaboration in Iceland. We are told in two lines that the idea (of deep drilling) has been implemented in Canada, West Germany, Japan, South Africa and the Soviet Union. And that is all. If this is a consequence of a growing nationalism in matters of energy and mineral resources, then it is a bad one, and it is to be hoped that any scheme ultimately approved takes note of the international dimension. □

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*Continental Drilling (ed. by Shoemaker, E. M., Carnegie Institution of Washington, 1975).

ZWO: past present and future

Arie de Kool reports from Rotterdam on the evolution of the Netherlands Organisation for Pure Scientific Research, which is 25 years old.

WHEN the first council of the Netherlands Organisation for Pure Scientific Research (ZWO) was ceremoniously installed on May 31, 1950, the organisation had already worked for a couple of years, and had thus gained considerable experience. Ever since, it has been held in general esteem, even by those who have not been entirely happy with what the ZWO did. Even they will readily admit that the ZWO did a great deal and that it more or less shaped the face of Dutch science. This year, a quarter of a century later, the ZWO installed its fifth council—during an equally solemn session, attended by Queen Juliana, members of the government and hundreds of prominent scientists—but it was generally felt that this might well be the last one.

As official proposals now stand, the ZWO is to merge with several other institutions to form the Council for Scientific Research (RWO). As far as Dr J. H. Bannier, the present ZWO director, is concerned, this would be just fine, with some important provisos.

- The RWO should have its own independent funds, with which it could react quickly to any request for support, without any of the red tape involved in government ministeries.
- The RWO should be able to manage research (mainly) by progress control.
- RWO should have far better formal contracts with universities than is provided for in the government proposals. (The ZWO has two representatives for each university and one for each specialised school, such as the institutes of technology, on its council, and even that is not found quite sufficient.)
- The RWO should provide interdisciplinary contacts.
- The RWO should not manage more than 20% of the total budget for fundamental research, albeit in coordination with the other, equally independent channel for money supply —the government through direct support for universities and other institutes.

It is difficult to determine just when the idea of an organisation for pure scientific research was born. Some trace it back to the German concentration camp of St Michielsgestel in the Netherlands, where a considerable number of prominent Dutchmen were kept in (relatively decent) custody—partly as hostages, partly to keep them from organising anything subversive.

This camp was a breeding ground for ideas on how to change the country at the end of the war. But when the concentration camp was liberated, the idealistic inmates found the country still at war and themselves without the means to implement what they had planned in such gloomy conditions. When at last the war was over, it took some time to find out in how much of a shambles the country was, and even more time to realise the size of the gap between the Netherlands and countries that had not been occupied.

Once this had been established, however, things proceded at a fantastic pace. Four months after the war, a new, more or less provisional government took over power from the military, and scientists started to meet again. They decided that a prominent member of their community should go to the USA and find out what the state of science was and how that country had managed such scientific marvels as the atom bomb. This scouting job was assigned to Professor Vening Meinesz, a geophysicist.

Physics seemed the most promising field, together with mathematics (the first computers had been built). A month after Meinesz returned, in January 1946, a mathematical centre was founded in Amsterdam, and in March 1946 the Minister of Education and Science, Van der Leeuw, one of those detained in St Michielsgestel, was instrumental in establishing a Foundation for Fundamental Research on Matter (FOM).

This foundation was immediately successful, and has been ever since. It has become the model for virtually all ideas in the Netherlands on how to organise science. Even the fiercest critics of the organisation still maintain the basic model when they propose their alternatives.

The main instrument is the 'working community'—in which each particular speciality is discussed. So there is a working community for solid state physics, one for nuclear physics, one for high energy physics, one for plasma physics and so on. The working communities submit their plans to the FOM and the council then decides who gets what, basically on scientific merit only; in practice, however, scientific, technological and social merits cannot always be clearly separated.

By August 1946 a report was brought out proposing the establishment of an overall organisation for scientific research, working essentially along the same lines as the FOM but on a larger scale.

There had been elections in the meantime, however, and a new government had come to power; the new Minister of Education and Science seemed to be far too busy restoring the ministry to its pre-war status to have either time or interest to devote to science and its organisation.

It took about 6 months to get any reaction at all, and he finally conceded that if those scientists absolutely wanted to, he would not keep them from continuing their study on how to organise science.

The scientists went further than that. They set up a provisional organisation for the stimulation of science by judgment (and selective support) of proposals. The organisation had one foot in the ministry itself: its secretary (a gift from the former minister) was a public servant who had had a scientific education and who headed the office of higher education and science—J. H. Bannier.

After a year the first chairman of the committee (director of the organisation) accepted a professorship and left, and by that time it had become a matter of course that Bannier would take his place. It took two more years, however, for the government to pass a law to establish the ZWO officially. During the first four years it worked merely as an advisory body, for the government did not really feel like handing out large amounts of money to scientists to divide among themselves. This meant that for every penny earmarked for the support of some project, at least 25 signatures from different departments were needed before the money could actually be made available.

In the first year the ZWO received the full amount of Dfl 1.5 million ($400,000 or £130,000 at that time) More than half of this was earmarked for the FOM which, with some other foundations, was made a sort of independent subsidiary of the ZWO Last year, the ZWO administered some Dfl 125 million, the equivalent of $50 million or £20 million. Dutch government expenditure on research and development totals some Dfl 2,000 million ($800 million or £350 million), while the total research and developmen expenditure of the Netherlands i about twice that amount. Universitie are thought to spend some Dfl 1,000 million on research, but nobody i very certain about that.

In practice, the ZWO's influence i much greater than it might seem simply from these figures. In physic there is hardly any research done outside the coordinating influence of on of the working communities of th ZWO's 'subsidiary', the FOM. This i not quite the case in other discipli although the ZWO subsidiary FUNGO (Foundation for Fundamental Medical Research) claims that it really controls about twenty times the amount of research it pays for. Other ZWO foundations (for pure chemistry, biological research, biophysics, and some smaller areas) claim a similar, but smaller, influence.

The method followed is quite simple. The ZWO (or one of its foundations) may support a project by providing one extra man, or a piece of costly machinery, or just the costs of publication. In the total project budget this may be a rather minor point, and a condition is, of course, that the project be brought in for assessment and coordination.

Lately, however, there has been some criticism of this method. Not on scientific grounds, for almost everybody agrees that the system is working well (although some would want more openness and some would go for a more democratic procedure in appointing the judges). But the method implies the existence of temporary positions—appointments of scientists for the duration of just one project.

Temporary positions were generally accepted for as long as scientific posts were plentiful. Doing a project for the ZWO (often as a way of obtaining a PhD) could often help a person to find a more permanent position. Now that the market is getting really difficult, people are wondering if temporary appointments are still socially acceptable. The ZWO does not want to behave in a socially irresponsible way, and the organisation has set out to ensure that its employees receive a normal income when unemployed after a temporary appointment. But according to Professor R. van Lieshout, now managing director of the ZWO and professor of physics at Amsterdam, the problem has not yet had its full impact. Unemployment is most serious where it has always been a problem—among social scientists mainly—and physicists can still find employment. But Professor van Lieshout does not have many words of comfort for ZWO scientists generally. His message is: "I advise most people on a temporary contract with the ZWO to find themselves a permanent job as soon as possible." ☐

international news

Australian science in turmoil

from Peter Pockley, Sydney

Within 10 extraordinary days, the mood of science in the political fabric of Australia has passed from one of constructive optimism to destructive despair. The change has mirrored an upheaval within the Labour government precipitated by a massive reshuffle of Cabinet portfolios by Prime Minister Gough Whitlam announced late on the night of June 5. Australian scientists have been shocked to find their interests and their principal research organisation, CSIRO, subject to a totally unexpected battering. They have a new minister, Mr Clyde Cameron, who came into the portfolio with a public scream, and CSIRO is subject to surgery without the courtesy of consultation.

On May 29, only seven days before the Cabinet reshuffle, the long awaited Australian Science and Technology Council (ASTEC) held its inaugural meeting in Canberra. The formation and role of ASTEC had been announced by Mr Whitlam at the big ANZAAS Congress in Canberra in January but virtually nothing of significance followed the ASTEC announcement until late May. The then Minister for Science, Mr Bill Morrison, had expected ASTEC to get underway rapidly after the ANZAAS announcement, but as seemed to happen with many things in his 2½ years of scientific jurisdiction, matters ground along slowly.

It took four months to settle the interim membership of ASTEC ("interim" because it has been established before the passage of legislation to give it statutory independence). Rumours had flown around the scientific community of the difficulty Mr Morrison was said to be having in getting the agreement of people to serve. Probably more to the point was Mr Morrison's lack of regard for many of the longer standing leading figures of Australian science, who, he was heard often to say, kept cropping up repeatedly on government committees. As a result he was not disposed to appoint to ASTEC many of the names put up, for instance, by the Australian Academy of Science.

The final 12 nominations, although lacking weight in some fields, did show some ingenuity in the balance of interests represented, thereby lending credence to Mr Morrison's intention that ASTEC should have an influence on the wide range of government policies on which science and technology have a direct, if hitherto unrecognised, impact. The crucial post of chairman went to Dr J. A. Louis Matheson, an engineer by background, who will retire as Vice-Chancellor of Monash University at the end of the year, when he will be able to take up his ASTEC duties on a full-time basis.

The other part-time members can be classified in various ways—six university professors, two technologists, a managing director of a technological firm, and a trade unionist. The appointment of a philosopher, Professor John A. Passmore of the Australian National University, was an imaginative move (he had taught Mr Morrison years earlier) and the inclusion of Professor Sol Encel, a sociologist at the University of New South Wales and one of Australia's few students of science policy as a discipline, has been an insurance against ignorant parochialism.

The Australian National University, established in the national capital, Canberra, has four members on the council. The leading medical researcher and administrator, Professor Gustav J. V. Nossal, Director of the Walter and Eliza Hall Institute of Medical Research in Melbourne, will be sure to keep ASTEC interested in the strategy of Australian medical research, an area which earlier had been thought likely to be specifically excluded from ASTEC's purview.

The total number of members announced in mid-May was 11, not the 12, and this shortfall of one brought out the first sign of political trouble ahead for science in Australia. In announcing his 'First Eleven', Mr Morrison told the world that the Premier of the State of New South Wales, Mr Tom Lewis, had refused to make available the services of Mrs Marlene Brell, a teacher, nutritionist, and (above all, for Mr Morrison who espouses both the consumer and female causes) a woman and a prominent consumer advocate. Mrs Brell had the misfortune to be employed under a state authority, and just now there is a spate of bitterness between the Liberal–Country Party government of New South Wales and the Labour government in Canberra. A futile slanging match followed between the politicians, and ASTEC still has only 11 members.

In a constructive move to keep science away from party politics in Canberra at least (a wise, if rare, action in the light of the fractious mood of the national Parliament where the Opposition uses its majority in the Senate to defeat much of the government's legislative programme), Mr Morrison invited his opposite number in the Liberal Party to the first ASTEC meeting. Mr Eric Robinson had succeeded Mr Tony Street as Opposition spokesman on science two months earlier, following a leadership struggle in the Liberal Party which saw Mr Malcolm Fraser, a former Minister for Education and Science, emerge as the new Leader of the Opposition. Mr Street had drafted the Liberal Party's first statement on science policy in March (Liberals, including Mr Fraser as minister, had previously denied consistently that a policy for science was even capable of definition); this differed little in detail from the Labour platform.

The omens for a bipartisan approach to science were good. And necessary, too, for Mr Morrison's lack of real conviction that science needed increased support had resulted in a slow but serious downward drift in public support for research and development compared with a massive injection of government funds into other areas such as social services, health services, education and urban development. Mr Morrison's belief that there are no votes in science had led him to look elsewhere for political kudos; he found some by grafting the government's nascent interest in consumer affairs on to his portfolio, and he found some more by maintaining his vestigial responsibilities under Foreign Affairs for the nearly independent nation of Papua New Guinea and an assistant role in Defence.

With ASTEC one week old, Mr Morrison achieved his ambition for promotion out of the lowly Science portfolio to Defence. The reshuffle which allowed his move came about through the resignation of the Minister for Defence, Mr Lance Barnard, to take a diplomatic post.

Mr Whitlam and his government, which scraped back with a majority of five in an election 12 months ago, were plagued with inept performances by several of the over-large 27-man Cabinet. Mr Barnard's resignation gave him the chance to promote the able ministers and demote or transfer to supposedly harmless duties the incapable and the stubborn. One in the last category was Mr Clyde Cameron, a determined and capable Minister for Labour and Immigration, who nevertheless fell foul of Mr Whitlam through his intransigent support of inflationary wage demands. One by one, the Prime Minister placed his ministers in new slots (he has no choice over the people in the ministry who are elected by the whole Labour Caucus); the last slot left was Science and Consumer Affairs (a new portmanteau title, thanks to the outgoing Minister, Mr Morrison) and the last minister he contacted was Mr Cameron. His old portfolio had already been allocated to another, and Mr Cameron refused to serve. He held out for the best part of the day while the other ministers were sworn in; the Prime Minister began formal arrangements with the Governor-General to have Mr Cameron dismissed. The relucant and unsmiling Mr Cameron eventually caved in and accepted. No Australian minister responsible for science had achieved such dramatic publicity. And Australian science has never received such unwarranted notoriety.

● Next, CSIRO was thrown into turmoil. Radical action of a kind previously uncharacteristic of Australian scientists, and CSIRO ones in particular, has taken over from their normally very private and cautious approach to political matters. On June 13 a series of unprecedented stop-work meetings were held by CSIRO staff around Australia.

The precipitating cause of the trouble was a sentence at the very end of the Prime Minister's press statement issued late on June 5 in which the new ministry was announced. At that time the Cameron controversy was still unresolved, and because several other senior ministers had their wings clipped to fit more junior posts, the Canberra press had time neither to read nor report the couple of pages at the back of the press statement which announced "The Department of Minerals and Energy will take over responsibility for the Minerals Research Laboratories and Solar Energy Studies Unit".

Nobody in the press gallery noticed that this meant a carve-up of CSIRO under which these laboratories and unit were developed from scratch. Wha is more extraordinary is that the Cha

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In a move which caught space scientists and NASA officials by surprise, the House Appropriations Committee last week voted to decimate the budget for the Venus Pioneer programme, which is one of NASA's priority planetary missions for the late 1970s. The committee has left only enough money in the budget to "maintain a management capability", and recommended that work on Venus Pioneer be deferred for a year while the mission's priority is re-examined. Specifically, the Committee suggested that a choice may have to be made between the Venus mission and the Large Space Telescope (LST), one of NASA's major astronomy programmes.

Before the committee's recommendation is put into effect, it must be approved by both the House and the Senate. The House is likely to follow suit in view of the empty rhetoric being voiced in Congress about the need to reduce federal spending, but NASA officials are trying to persuade the Senate Appropriations Committee to leave the mission intact. At this stage, it is uncertain whether they will succeed, but the immediate outlook for the Venus Pioneer programme should be considered dim.

Scheduled for launch in 1978, the Venus Pioneer mission would consist of two spacecraft, the first of which would study the planet from a highly elliptical orbit while the second would send a number of probes into the atmosphere. It would build extensively on the Mariner 10 results.

The House Appropriations Committee suggested that the mission should be deferred (the next launch window would be 19 months later) "to permit a budget priority decision in 1977 between the Large Space Telescope and Pioneer Venus". The committee made that recommendation because "some astronomers have been critical of NASA's Space Science programme because they contend that a disproportionate level of NASA dollars have been used on planetary astronomy missions, while little or no funds have been allocated to deep space astronomy which is the mission of the Large Space Telescope".

The LST, which was accorded top priority in a report issued earlier this year by the Space Science Board of the National Academy of Sciences, would be a large optical telescope placed in Earth orbit by the space shuttle early in the 1980s. Although some questions have been raised about its priority, debate has mostly been on the merits of the LST compared with a mission to Jupiter and Uranus. To pit the LST against the Pioneer Venus mission, a NASA official remarked last week, "opens up a whole new ball game".

Colin Norman, Washington

man, Executive and staff of CSIRO did not learn about their loss (let alone have any forewarning of the announcement) until the next day, June 6, a day on which their reluctant new minister was totally involved with his own fate

The Minister for Minerals and Energy is Mr Rex Connor, who with his Departmental Head, Sir Lenox Hewitt, is tough, intractable, inaccessible and uncommunicative. The main scientific bodies under the Minerals and Energy portfolio are the Australian Atomic Energy Commission (AAEC) and the Bureau of Mineral Resources, both worthy but lacking any scientific high-fliers. Since coming to office, Connor and Hewitt have brooked no opposition in building their empire of bureaucracy, legislation and influence; lately they have been helped by their highly political scientific adviser, Professor Harry Messel, no mean empire builder himself as Head of the School of Physics at the University of Sydney. Messel was recently appointed a part-time Commissioner on the Atomic Energy Commission and is being tipped as the next Chairman. Connor has had ambitions to turn the AAEC into an energy research body not specialising in nuclear power, but lately he has appeared almost obsessed with the prospects of Australia developing a massive, indigenous industry for uranium enrichment—under his control, of course—and there have been broad hints, with no specific evidence in support, that Australia has developed its own enrichment technology.

The tight-fisted control and the secretive style of the Minerals and Energy operations, at both the political and bureaucratic levels, have been privately deplored by CSIRO and academic scientists. Yet, for over two years CSIRO has officially been trying to make effective contact with Minerals and Energy for collaborative projects on energy research; most of their efforts even to establish contact have been ignored.

These facts have compounded the shock felt throughout CSIRO by the astoundingly successful raid on their domain carried out at a time of such political confusion. The raid had been planned over many months—at least since the appearance of the OECD report in mid-1974 on Australian science policy which had criticised Australia for having no effective energy policy, a statement to which Connor took furious exception. The gentlemanly scientists of CSIRO simply had erected no defensive barriers to deter the raiders from implementing their plot. The Chairman of the CSIRO, Dr Jerry Price, has effectively put his job on the line by calling a press conference in which he roundly condemned the government's move. He has the support, though, of the CSIRO Advisory Council, a body of eminent outside experts, who in their first-ever emergency meeting unanimously criticised the Prime Minister and are seeking an audience with him "to propose alternative ways in which the objectives of the government in regard to minerals and energy could be better achieved than the present proposal". Dr Price said the carve-up of CSIRO could mark the beginning of the end of the 7,000-strong organisation as an integrated operation and also as one with a high level of purposeful morale.

The staff of CSIRO have become politically active as never before. Petitions, telegrams and representations to politicians, mass meetings, a one-day strike (done by the rather quaint method of staff applying *en masse* for a day's leave, with the concurrence of their chiefs) and press publicity have taken over from research for the moment. The Liberal Opposition has rediscovered science as an issue to use against the government. The Chairman of ASTEC, Dr Metheson, his council barely functioning, has protested at being bypassed; the questions involved in the organisation of energy research should have been examined by ASTEC if this innovatory organisation is to have any meaning or influence. ☐

More armament and no disarmament

from a correspondent

THE Stockholm International Peace Research Institute (SIPRI) published its Yearbook for 1975 last week. As usual, the 600-page volume catalogues the last twelve months' developments in world armaments and disarmament. As usual, it makes sobering reading.

The general picture emerging from the Yearbook is one of a world approaching the threshold of widespread proliferation of nuclear weapons. The Indian nuclear explosion in May 1974 was a dramatic demonstration of the ease and cheapness with which a country with a significant peaceful nuclear programme can produce nuclear explosives. (The explosion probably cost no more than $400,000, including the cost of the plutonium and the preparation of the test site.) At the end of 1974, 19 countries had operational nuclear power plants producing a total of about 73,000 MWe; and if present plans are carried out there will, in 1980, be 28 countries with nuclear power reactors capable of generating a total of about 300,000 MWe. Looking further ahead, by the year 2000 it is probable that the 1980 figure will have multiplied more than tenfold. The Yearbook draws attention to the amount of plutonium that these reactors will produce: 350,000 kg in accumulated stocks by 1980. This situation will give rise to two sorts of danger: first, manufacture of nuclear weapons by countries with plutonium, after the Indian example, and second, nuclear terrorism.

The recent conference held in Geneva to review the Nuclear Non-Proliferation Treaty (NPT) was intended—and completely failed—to strengthen that treaty, the only political barrier to proliferation. In the light of the failure of the conference, and given the relative technological ease with which nuclear weapons have been shown to be produced from peaceful nuclear programmes, there will be little incentive for countries with plutonium stocks to refrain from producing nuclear weapons if they perceive that these weapons would strengthen their security. Nuclear terrorism will be facilitated by the very amount of plutonium existing by 1980. Even if it is safeguarded with 99.9% efficiency, the weight diverted from its intended use will be enough to manufacture a bomb a week.

The threat of nuclear proliferation tends to obscure the fact that nuclear weapons and their delivery systems account for only about 10% of the total world military expenditure. When nuclear and conventional expenditures are considered together, the trend points to other causes for alarm. In 1974 the world spent over $210 thousand million on armaments: a sum approximately equivalent to the total income of the poorer half of mankind. For the first time, NATO and Warsaw Pact forces between them spent less than 80% of this amount. The areas whose shares have been rising over the last 15 years are the Third World in general (whose expenditure has more than doubled), the Middle East, which has recorded a fivefold rise, and China, whose share has doubled. Thus the figures show increasingly intense procurement of armaments by more countries. The arms trade is flourishing as never before. Between 1973 and 1974 alone it increased in value by 40%. In 1974, the Middle East was the recipient of more than half the total arms supplied. The Persian Gulf states received 27%.

The high cost of salaries for military personnel—a cost amounting to a third of total military expenditure—is making automated weaponry more attractive. The USA is developing a new weapon system which could provide a fourth component to the arms race, after strategic bombers, nuclear submarines and intercontinental ballistic missiles. The new system is the long-range cruise (non-ballistic) missile (LRCM). It can be launched either from submarines or from aircraft, and has both tactical and strategic capabilities. Its distinctive feature is great accuracy of delivery: an accuracy which is also being built into the class of conventional weapons known as precision guided munitions. Such munitions now include a whole range of air-to-air, surface-to-air, air-to-surface and surface-to-surface weapons. The conventional battlefield is taking on a new look with the development of automated aids such as night vision equipment, artificial sensors which locate targets, command and control, and information-processing systems, all of which are bringing the automated battlefield nearer and nearer.

Against this background of threatening proliferation, increasing militarisation and new weapons technologies, the Yearbook cannot point to any convincing measures of arms control or disarmament. The meeting between President Ford and General Secretary Brezhnev in Vladivostok in November 1974 set the limit of missiles with multiple independently-targeted re-entry warheads at 1,320: more than either side had deployed at the time of the accord. Presumably such a high figure was chosen so that each side could complete its planned deployments and still reap the political benefits of having arrived at an agreement. A similar situation obtained in the Threshold Test Ban Treaty, signed in July 1974 by the USA and the USSR. Each side undertook not to carry out any tests having a yield of more than 150 kilotons. Again, this limit is so high that it does not interfere with any tests intended by either side for any tactical and most strategic nuclear weapons at present deployed or even planned. The cynicism of the agreement is even more glaring given that refinements in accuracy are presently being pursued more enthusiastically than refinements in size. In fact, 1974 was a record year for the number of nuclear explosions touched off by all countries In all, 35 explosions were carried out: 20 by the USSR, 5 by the USA, 7 by France and 1 by each of the UK, China and India Treaties such as the Threshold Ban do nothing to limit the number of tests carried out by the signatory states, or to slow down the arms race.

SIPRI has a way of making the facts seem compelling. And yet there is more to international relations than just facts. The Yearbooks are invaluable for their collation and analysis of facts, but they do not analyse to what extent these facts influence the behaviour of decision makers. To enter this realm would admittedly be to go beyond the task SIPRI has set itself; and yet without it the Yearbook's pessimism occasionally seems too relentless. What is indisputable, however, is that the facts add up to more armament and no disarmament. And there is no change in sight. ☐

correspondence

In defence of MIT

SIR,—I am not often shocked by Nature, so all the greater the impact when that happens. It has just happened as the result of reading your leading article in the issue of May 22.

It is not one of your best, witness such an impenetrable sentence as: "And one thing that ought to have been learnt is that the isolation of scientific and other intellectual pursuits, particularly from bodies which claim to be representative, profits no-one and is a futile gesture in the face of strong regimes."

But this article ends with a clear and to me utterly reprehensible paragraph, saying that not all divisiveness stems from developing nations: "it should be added that some of the initiatives of the developed world seem only capable of generating bad feelings"; and then citing as its single example of such deplorable provocations that the Massachusetts Institute of Technology made acts of religious discrimination a cause for terminating a projected contract involving water desalination with Saudi Arabia. The *Nature* editorial ends: "Saudi Arabia can hardly welcome Jews with open arms; it surely behoves MIT to use restraint and not to trigger confrontation by insisting on sending them."

The relevant stipulations were in fact not written into the projected contract, but appeared in a covering letter from Jerome Wiesner, President of MIT. This stated in effect that under special circumstances either party could withdraw from the arrangement, and that MIT would feel obliged to terminate the contract if systematic discrimination in employment under the contract were exercised on the basis of race, colour, national origin, religion or sex. Such considerations govern all MIT employment, and are now required by American law. Saudi Arabia responded by withdrawing the proposal.

I am proud of MIT, which needed no prodding from the US government to adopt such practices since they are backed strongly by its faculty and students; and I am proud of the US government for having such laws.

But what are we to make of *Nature*?

Yours faithfully,
GEORGE WALD
Harvard University

Fossil footprints

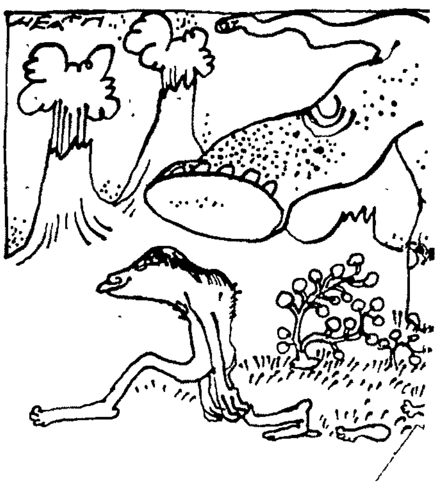
SIR,—The account of the finding of a fossilised human footprint in Turkey (*Nature*, April 17) would seem to indicate that fossil human footprints are now becoming scientifically respectable.

If this is so, readers might be interested in comparing those found in and around the Paluxy River, near Glen Rose, Texas, in rock usually identified as Cretaceous. The unusual thing there is that dinosaur footprints are found in the same strata.

These footprints have been shown in a well known film *Footprints in Stone*. Also, descriptions and comments have been given from time to time in the *Creation Research Society Quarterly*.

I have seen neither the prints themselves from Texas nor, of course, those from Turkey. On the basis of photographs, however, I can only say that the prints from Texas appear to be much better ones.

H. L. ARMSTRONG
Kingston, Ontario



news and views

Energy cost of food gathering

from Robert M. May

Recent changes in the cost of petroleum and other fossil fuels, allied with a growing awareness that these resources are finite, have prompted many studies of the economics of food production expressed in terms of energy rather than money: how many calories does it cost to put a calorie of food on the table? In this context, it is interesting to explore the energetic book-keeping of natural animal populations.

Lawton (in a chapter in *Resources and Population,* edit. by Benjamin Cox and Peel; Academic Press, 1973) has reviewed some of the data, gathered in the field and laboratory by various people, on the number of food calories obtained for one calorie of food-gathering effort. For the natural populations surveyed, this ratio runs from around 2 to around 20, with the typical value around 10. (For natural populations, with no energy subsidies, the ratio must obviously exceed unity.) This overall similarity in the ratio of energy gained to energy expended for the various species is true despite the fact that the absolute rates of energy expenditure during foraging vary over eight orders of magnitude, from the very high-cost techniques of hovering hummingbirds (with absolute rates of energy expenditure during foraging of between 16 and 33 cal min^{-1}), to the lower values for black bass (2–3 cal min^{-1}) and bumblebees (0.3–0.5 cal min^{-1}), to the extremely low values for the relatively inactive damselfly (around 5×10^{-5} to 5×10^{-6} cal min^{-1}). These absolute differences result both from the sort of foraging behaviour, and from the different body sizes of the animals. Lawton notes that energetically expensive food-gathering techniques in animals can only be applied to food sources which guarantee a high rate of energy return, and conversely: this fact probably accounts for the observed convergence in the ratio between energy gained and energy expended, independent of whether the basic strategy is 'high cost' or 'low cost'.

In simple human societies, obtaining their food without fossil fuel energy subsidies, the ratio of food calories gained to calories invested is similar to that which pertains throughout the animal kingdom. Thus the food of the islanders on Lamotrek Atoll in the South Pacific comprises root crops, coconuts, breadfruit, and animal protein in the form of pigs, fish, turtles and sea-urchins: this is gained by a mixture of hunting, gathering and farming, with an average ratio of energy gained to energy expended of around 20 (Odum, *Environment, Power and Society*; Wiley, 1971, after Alkire). Other data summarised by Odum and by Steinhart and Steinhart (*Science,* **184,** 307–316; 1974) suggest that hunter-gatherer societies typically average a ratio around 5–10, while shifting agriculture does better at around 20. A detailed study by Rappaport (*Scient. Am.*, **224**(8), 117–132; 1971) of the shifting cultivation practised by the Tsembaga in New Guinea gives a figure of 16. The wet rice agriculture practised for many centuries without energy subsidies in Indonesia, China, Burma and Thailand runs around a ratio of 10–50. In all, such 'primitive' cultures gain 5–50 food calories for each calorie of human energy invested, with a typical figure of around 10.

The analogous ratio in 'advanced' technological societies has been the subject of many recent analyses. The Steinharts have attempted to estimate the total number of fossil fuel calories invested, both upstream and downstream from the farm gate, in getting one calorie of food energy on to the contemporary US table. On the average, the ratio of calories output to calories input is 0.1. This figure, which differs by two orders of magnitude from that for animal and simple human populations, is generous in that it makes no allowance for private automobiles involved in getting the food home, nor for waste disposal (which currently engages 12% of all US trucks). Around the turn of the century the ratio was about 1, and it has declined steadily (averaging around 0.2 in 1940), partly in response to the growing per capita consumption of feedlot beef and other products high on the trophic ladder, and partly in response to ever more technologically intensive methods of farming. Thus even for corn, an ecologically efficient crop at the primary trophic level, current practices give an energy output/input ratio of 2.8 as the crop leaves the farm (Pimentel *et al.*, *Science,* **182,** 443–449; 1973).

In animal populations and most human societies, essentially everyone is involved in the business of getting food. (The social insects provide illuminating exceptions. But even here, the relative abundance of the various castes, and the division of labour among them, may be understood as minimising the total energy cost needed to maintain the society. The tentative beginnings of such 'ergonomic' studies are outlined by Wilson in chapter 18 of *The Insect Societies*; Harvard University Press, 1971.) In contrast, the increasing fossil fuel subsidisation of food production in the technologically advanced countries has been accompanied by significant changes in what we might call the 'ecological structure' of the work force. In the United States, farmers constituted 10% of the work force 50 years ago, and 2% today: one farmer feeds 50 people. Of course this figure is misleading, and when the people involved in all phases of the food supply system are added up they currently amount to around 20% of the work force (Pimentel *et al.*). This is still qualitatively different from essentially all previous animal (including human) societies, and leaves 80% of the work force free for less fundamental pursuits, some of which are necessary or at least useful (for example, building houses, reading *Nature*), others less so (for example, manufacturing bizarre products to gratify artificially created desires).

Many people have expounded the obvious implications of these facts in a world where fossil fuel supplies are finite and increasingly expensive: short-term problems in exporting technologically intensive agricultural methods to less developed countries; possible long-term problems in sustaining such energy subsidies in any society; artificial patterns of wasteful consumption in societies where only a relatively small proportion of the work force is deployed in truly essential tasks. But there has been very little detailed analysis of economic and social dynamics taking into account the basic ecological structure of the society. In other words, there is a lack of studies which draw a profound distinction between the demand curve for calories and protein, and the demand curve for plastic gee-gaws. Some light on the economic instabilities currently afflicting so many countries might be shed by the development of an 'ecological economics'. □

Band structure of solid inert gases

from B. L. Smith

THE inert gases have long been recognised as useful tools with which to develop the atomic theory of solids, liquids and gases. In the past twenty-five years much progress has been made, but there still remain considerable gaps in our understanding of these 'simple' systems.

In principle, properties of the solidified inert gases, including both thermodynamic and optical behaviour, can be determined by solving the Schrodinger equation, using the total Hamiltonian for all ions and electrons within the solid and their mutual interactions. In practice, the many body problem has to be reduced to manageable proportions by using approximations appropriate to the physical property under consideration. Thus virtually all the different approaches to the problem lead to essentially the one-electron approximation, that is, the band-structure formulation of the electron states, or to the exciton picture of excitations within the solid. A large number of band-structure calculations have now been carried out for the solidified inert gases.

The theoretical predictions can be compared with experimental data by measuring the reflectivity, absorption coefficient and the energy loss function. These, together with more recent determinations of photoemission, allow gap energies and electron affinities to be estimated. Accurate measurements are however extremely difficult because of various technical difficulties encountered in the experiments.

Photoelectron energy distribution curves for the solidified inert gases have recently been determined by N. Schwenter and coworkers at the University of Munich, in collaboration with E. E. Koch of the Deutsches Elektramen Synchrotron (DESY), at Hamburg (*Phys. Rev. Lett.*, **34**, 528; 1975). These measurements represent the first comprehensive set of experimental data for the actual band structure. They are important because they provide direct information concerning the structure of the valence bands, the influence of the conduction bands on the photoelectron energy distribution curves, and some details of electron–electron scattering processes. In particular, the data highlight the discrepancies which exist between the results of various band-structure calculations which have recently been reported.

Measurements were made on films of solid neon, argon, krypton and xenon, 20–100 Å thick, using the DESY synchrotron as a broad-band vacuum ultraviolet light source. The excitation energy was varied continuously between 8 and 30 eV with a typical resolution of 200 meV. Because the films were very thin, charging of the samples—one of the major problems encountered when investigating photoemission of insulators—was kept to a minimum.

The results provide a wealth of information about the band structure. In the first place, the vacuum level E_V is obtained directly and unambiguously for each solid. These values, combined with data for the gap energies E_G determined from absorption and reflection measurements, allow the electron affinities $E_A=E_V-E_G$ to be estimated and compared with the results of earlier photoelectron yield determinations. For example, E_A for neon is found to be both large and negative (-1.4 eV), which confirms the negative value predicted theoretically, and is consistent with the trend observed experimentally for the other inert gases.

Above threshold, the width of each energy distribution curve increases with increasing photon energy until the photon energy is sufficient to excite electrons from the bottom of the valence band. In this region the width of the curve corresponds to the width of the valence band and is independent of the energy of the exciting photons. Photoemission thus allows the valence band width to be estimated. Schwenter found that the widths of the energy distribution curve increased systematically from neon to xenon as might be expected from the spin-orbit splitting, and were larger then the widths predicted by most band-structure calculations.

There are some interesting anomalies which arise when examining the various band-structure calculations. For example, relativistic calculations for krypton result in a total band width that is 0.5 eV smaller than that observed (2.3 eV), whereas non-relativistic calculations exceed the experimental width by similar amounts. The best agreement between theory and experiment is obtained by simply adding the spin-orbit energy to the width of non-relativistic bands obtained by Kunz and Mickish (*Phys. Rev.*, **B8**, 779; 1973), but this procedure is somewhat dubious.

A hundred years ago

THE preparations for the Geographical Congress in Paris are being actively completed. The large map of France executed by the staff officers will be exhibited, all the sheets having been joined, thus forming one continuous sheet of paper of immense size. The map will be exhibited at the Tuileries in the Salle des États. It will be photographed by the microscopical and panoramic process.

from *Nature*, **12**, 174; July 1, 1875

Three maxima are noted in typical photoelectron energy distribution curves for xenon but these are not detected for the other solidified inert gases, presumably because of the overlapping caused by the smaller spin-orbit splitting and smaller total band width in the other solids. The shape and relative intensities of the curves are found to depend strongly on the exciting photon energies. Using the experimentally determined parameters for the valence bands, these variations can be used to obtain estimates for the structure of the density of states in the conduction bands. For example, the first maximum of the density of states can be deduced, which corresponds to flat regions of the lowest conductance band. The experimental data thus yield new and important information about the structure of the conduction bands, hitherto unobtainable by other methods.

How do the above calculations and measurements relate to the cohesive energy in the solidified inert gases? It is known that one-electron energies, as obtained for example from Hartree–Fock calculations, over-emphasise Coulomb and exchange terms. More importantly, band energies do not fully include electron–electron interactions. Since electron–electron correlations would be expected to be important in the case of the inert gases, these calculations need some modification to take these effects into account. For example, the basic Hartree–Fock assumption that the ground state wave function can be described as a single Slater determinant of one-particle wave functions can be modified by including excited one-particle wave functions in the combination. But this and other approaches soon run into computational difficulties and still no entirely satisfactory method exists for solving the problem.

Eisenschitz and London carried out the perturbation calculation in 1930 which established the attractive part of the force between two non-polar atoms—the Van der Waals or dispersive term. In the following decade it was confidently predicted that because the atomic polarisability had been linked to interatomic forces, once the structures of the solidified inert gases were established, it would be a relatively simple matter to relate the atomic properties to the bulk thermal and mechanical behaviour.

The past twenty-five years have proved the contrary. Although some very elegant experiments have been performed and a great number of calculations have been carried out, the details, both theoretical and experimental, still prove to be very elusive.

Diamond in a kimberlite xenolith

from G. Malcolm Brown

MOST of the world's diamonds occur as sparsely distributed crystals in the rare, ultramafic potassic rock called kimberlite. The rock is found in pipe-like bodies that reach the present Earth surface, as for example in the major diamond localities of South Africa and Siberia. There is no doubt that the kimberlite material was injected into the crust from deep levels of the Earth, aided in many cases by the explosive forces of a high gas content. The kimberlite matrix has apparently crystallised from a liquid magma, and the fascinating array of rock fragments (xenoliths) embedded in the matrix bear testimony to the source of the magma. These xenoliths are mainly garnet lherzolites, rocks consisting chiefly of garnet, olivine, clinopyroxene and orthopyroxene crystals. Such ultramafic rocks are now widely accepted as being most closely representative of the composition of the Earth's upper mantle. Hence the intrusion of kimberlite has resulted in the transport, to near-surface environments, of parts of the Earth that are otherwise invisible except by indirect geophysical measurements.

Diamonds are not a geologist's best friend. Most minerals occur in a textural association with other minerals such that constraints can be placed on the physico-chemical properties of the whole rock assemblage, and much information can thus be gained about the processes involved in their origin. In contrast, most diamonds occur within the heterogeneous, often fragmentary matrix of the kimberlite pipes and the critical information on temperatures and pressures (and thus depths) of origin can only be inferred from studies on the associated, but generally diamond-free fragments of ultramafic upper mantle (lherzolitic) rock. Another complicating factor is that whereas lherzolite xenoliths occur in many volcanic lavas generated from the upper mantle, they are diamond-free. Hence the genesis of diamond has seemed to be associated specifically with the kimberlite matrix of the diamond pipes, rather than with upper mantle xenoliths as a whole.

Dawson and Smith (*Nature*, **254**, 580–581; 1975) have therefore made an important recent discovery. They describe the finding of a small diamond crystal (0.8 × 1.4 mm) in a thin section preparation of a mica-bearing garnet lherzolite xenolith from a kimberlite pipe (diatreme) in Lesotho. C. F. Davidson once wrote that "the richest kimberlites . . . carry one part diamond in 10^7 of rock, and the average is one in 10^{10} or less. So the chances of finding a diamond in a thin section are somewhat around the cube of the probability of finding the needle in the haystack" (in *Ultramafic and Related Rocks*, edit. by Wyllie, 302; Wiley, 1967). Small wonder, therefore, that diamonds are rarely detected other than by the crushing and sorting of huge volumes of kimberlite rocks. Dawson and Smith refer to the few previously recorded occurrences of diamond in eclogite and garnet serpentinite xenoliths from kimberlites, but those xenoliths are of slightly lesser relevance to an upper mantle source material.

The discovery of diamond in the lherzolite permits mineralogists to use the lherzolite minerals in order to estimate the pressure (*P*) and temperature (*T*) of formation of the included diamond crystal. In fact, studies of this type have been pursued vigorously since 1973, stemming especially from original work by Boyd and coworkers on the pyroxene mineralogy. Unfortunately, despite a number of sophisticated studies on the phase equilibria and related thermodynamic calculations, the topic is still plagued by doubts as to the dependability of certain data used in the geothermometry and geobarometry estimates. Each new study adds further refinements, though the basic concepts are not questioned, and it is premature for readers to assume that the *P–T* values given by Dawson and Smith are better than a very broad estimate.

They give valuable mineral analyses and conclude that the assemblage equilibrated at 1,050 °C, at a pressure of about 4.6 to 5.2 kPa (that is, about 150 km depth). There are, however, several other lines of evidence that need to be considered. Graphite, the other form of carbon in igneous rocks, occurs in some garnet lherzolite xenoliths in kimberlites, as they mention, so the *P–T* curve for the graphite–diamond transition is relevant in the sense that proximity to this phase boundary is likely. Also, studies of mineral inclusions in diamonds have yielded important data for *P–T* estimates.

A method of estimating *P–T* conditions that is more rigorous than analogy with simple synthetic systems is more than overdue. Such an approach, using thermodynamic parameters to formulate a geothermometer based on the iron–magnesium exchange reaction between olivine and calcium-rich clinopyroxene, has recently been developed by Powell and Powell (*Contr. Min. Petrol.*, **48**, 249–263; 1974). They examine *P–T* conditions for inclusions in diamond and for ultrabasic xenoliths in kimberlites, and the related studies are cited. Where the *P–T* lines are drawn for those assemblages, they intersect the graphite–diamond transition line at about 1,250 °C and 4.5 kPa. Thus the pressures inferred by Dawson and Smith are corroborated, but the temperature discrepancy is 200 °C. In fact, plotting of the Dawson and Smith data (at 1,050 °C) on the Powell and Powell diagram would suggest equilibration improbably close to the wet pyrolite solidus.

Reference to other methods of estimating *P–T* conditions, which may or may not be proven better, is made merely to stress the need for caution in accepting specific values at this stage in a rapidly developing science. The major contribution by Dawson and Smith remains an exciting one. They raise other interesting points regarding the stability of phlogopite mica, diamond growth habits, and the possible genesis of graphite by breakdown of diamond. Above all, however, they remind us that the processes of generation of diamond are interwoven with the processes of upper mantle dynamics and magma generation at around 150 km depth in the Earth.

A probe probed

from E. G. Richards

To investigate the structure of DNA you need a probe that will interact with the macromolecule. Electromagnetic radiation has long been a favourite but various chemical reagents are strong contenders. Of these, the simplest is the proton and the next most simple is possibly formaldehyde but in order to interpret the interactions you observe you need to know in detail what is going on. This has hitherto been more than a little obscure in the case of formaldehyde, a matter which has now been put to rights by two elegant and exhaustive papers by McGhee and von Hippel (*Biochemistry*, **14**, 1281 and 1297; 1975).

To begin with, formaldehyde can react with both the exocyclic amino groups of A, C and G residues and the endocyclic imino groups of T and G (and U and I). In each case the result is the substitution of a hydrogen atom by a methylol ($-\mathrm{CH_2OH}$) group. In the case of the reaction with the amino group, a monoadduct or a diadduct may be formed according to whether just one or both hydrogens are substituted and it would seem that the ghost of the Schiff base has been laid. A further wrinkle in the exocyclic reaction is provided by the fact that the amino group is coplanar with the heterocyclic ring so that there is the possibility that the monoadduct can exist in two isomeric forms only one of which would interfere with the formation of Watson–Crick base pairs. Experiments with various methylated bases suggested that the more stable isomer precluded base pairs in the case of A and C but not in the case of G residues.

The equilibrium constants for the formation of the various adducts were measured using spectroscopic methods and employing several clever tricks to analyse the data. The formation of the monoadduct in the exocyclic reaction yielded values in the region of 13 M^{-1} while the formation of the diadduct from these gave values some 35 times smaller. The endocyclic reaction gave values of about 2 M^{-1}. Both sorts of reaction were exothermic with enthalpy changes in the range −2 to −6 Kcal mol^{-1} according to the compound studied and the nature of the reaction. Thus at 23 °C in 1 M formaldehyde, for example, deoxyadenosine would exist at equilibrium as 25% diadduct, 70% monoadduct and 5% unreacted nucleoside. At higher temperatures one should expect less adduct.

The forward and backward rate constants for adduct formation were also studied. The forward constants for the formation of the monoadduct in the exocyclic reaction turn out to be in the range $0.5–13 \times 10^{-4}$ M^{-1} s^{-1} which is rather slow. Thus the half life of decomposition of an adenine adduct at 0 °C is about 60 h so that dialysis of nucleic acids for a day or two in the cold would be quite inadequate to remove reversibly bound formaldehyde. The rates of these reactions were found to be independent of *p*H and remarkably insensitive to the addition of compounds such as tetramethyl ammonium chloride, sodium perchlorate or dioxane which perturb the melting of DNA. In another nice point a good linear relation was demonstrated between the logarithm of the rate constant for monoadduct formation of the *pK* of protonation of the amino group for a long series of substituted anilines and similar compounds. Extrapolation of this relation suggested that the *pK* of protonation of the amino group in adenine and cytidine was in the range −2 to −3.

The endocyclic reaction was found to be faster by two or three orders of magnitude and, moreover, *p*H dependent. At *p*H 7 there was a similar linear relation between the logarithm of the rate constant and the *pK* for several nucleic acid base derivatives and also, in the case of 5′TMP, with *p*H.

All these data are interpreted in terms of a mechanism wherein a formaldehyde molecule approaches a base in a direction perpendicular to its plane. In the case of the amino group reaction, an intermediate tetrahedral zwitterion is formed which then reverts to the planar adduct. In the case of the imino group reaction the formaldehyde reacts with the negatively charged ionised form and the charged intermediate is then rapidly protonated to form the neutral adduct. In either case the reaction is expected to be inhibited by base stacking such as would be present in a double helix.

Some of the implications of these results to the study of the structure and kinetics of unfolding of DNA are obvious but the further instalments along these lines promised by the authors will be eagerly received.

In a paper containing less physical chemistry but more immediate relevance to nucleic acid structure, Rhodes (*J. molec. Biol.*, **94**, 449; 1975) describes the reaction of methoxyamine and carbodiimide with yeast tRNA$^{\text{phe}}$. The first of these reagents is specific to C and the other to U and G; contrary to some earlier reports it was also found to react with dihydrouridine. Using fingerprinting techniques she was able to determine which bases in the sequence were attacked and it is gratifying that the results are wholly consistent with the three dimensional structure of this tRNA species as recently revealed by X-ray diffraction. This was so even in the presence of spermine as used in the crystallisation brew.

It has always been a worry that the packing of the tRNA molecules into the crystal lattice could result in their distortion into biologically irrelevant shapes. Rhodes's work goes some way to allaying such anxieties.

Xeroderma pigmentosum and light-induced cancer

from F. Giannelli

THREE recent papers in the *Proceedings of the National Academy of Science* have set the spotlight on Xeroderma pigmentosum (XP), a rare recessive disease which seems to hold the answer to many questions on the genetics and physiology of DNA repair in man and on its role in cancer and development.

Patients with this disease show, soon after birth, hypersensitivity to sunlight gradually followed by regressive changes and multiple recurring neoplasms in the exposed parts of the skin. They may also present neurological abnormalities which in approximately 14% of the cases are grossly incapacitating and progressive.

In 1968 Cleaver discovered that XP cells are deficient in excision repair, a DNA repair system which, by a series of enzymatic steps, excises DNA lesions caused by ultraviolet light or similar mutagens, and replaces them with patches of DNA newly synthesised using the undamaged strand as a template. It seemed reasonable at that time to suggest that unrepaired DNA damage was a sufficient and necessary cause of light-induced cancer. Since 1971, however, patients with typical features of XP but normal excision repair (XP-variants) have been observed and, it seemed, therefore, appropriate to retreat to a less dogmatic view and postulate simply that unrepaired damage was probably a sufficient though possibly not a necessary cause of actinic cancers. For four years the XP-variants have been an uncomfortable puzzle, but now Lehmann *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 219; 1975) have succeeded in showing that cells from such patients are defective in a process (post-replication repair) which enables normal cells to avoid reproductive death by overcoming the obstacles to DNA replication caused by uncorrected DNA damage. Lehmann *et al*'s (1975) findings are in keeping with the observation that XP-variants are less efficient than normal cells in the reactivation of ultraviolet-irradiated infecting adenovirus 2 (Day, *Nature*, **253**, 748; 1975) and clearly indicate that the XP phenotype is the direct consequence of a DNA repair defect.

As so often happens in science, when one problem is solved, another appears.

For many years it has been thought that placental mammals, and man in particular, are deficient in enzymatic photoreactivation—a repair process catalysed by an enzyme which, if activated by light of the appropriate wavelength, splits the covalent bonds whose formation between adjacent pyrimidines of the same DNA strand is induced by ultraviolet irradiation (Cook, J. S., in *Photophysiology*, edit. by Giese, A. C., **5**, 191; 1970; Trosko, J. E., and Isoun, M., *Int. J. Radiat Biol.*, **18**, 271; 1970). Recently, however, Sutherland and collaborators in a series of papers have presented evidence to show that photoreactivating enzyme is present in human leukocytes and fibroblasts, plays a part in the reactivation of infecting ultraviolet damaged Herpes Simplex virus, is active also on the DNA of living cells and is absent or markedly reduced in excision proficient and defective XP cells strains (*Nature*, **248** 109; 1974; *Biochemistry*, **13**, 4710; 1974 *Nature*, **254**, 627; 1975; *Proc. natn Acad. Sci. U.S.A*,. **72**, 103; 1975).

The findings of Sutherland *et al.* in XP are unexpected and pose many intriguing questions. For example, is the defect in photoreactivation secondary and incidental to defects in dark repair or does it play an important part in the development of the XP phenotype, a view favoured by Sutherland *et al.* (*Proc natn. Acad. Sci. U.S.A.*, **72**, 103; 1975). If the latter were true an inverse relationship could be expected between the severity of the photoreactivation and the dark repair defects observed in different patients but this is not yet borne out by the available data. The constant association observed between photoreactivation and dark repair defects in XP is difficult to explain in simple genetic terms. For example, the hypothesis that the two defects are inherited as independent mutations could make sense only if defects in one repair system alone were insufficient to produce the XP phenotype and if the mutations affecting photoreactivation and both dark repair systems (excision and post-replication) were very closely linked. This seems unlikely especially if one considers the genetic heterogeneity of excision-defective XP. So far five complementation groups and two types of XP showing distinct complementation kinetics have been recognised and it seems possible that as many as five loci are involved in this disease in spite of the apparent similarity of the biochemical defect in the different complementation groups (Kraemer *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 59; 1975; Bootsma *et al.* in *Molecular Mechanisms for the repair of DNA*, edit. by Hanawalt and Setlow; Plenum Press, New York, in the press; Giannelli and Pawsey, *J. Cell. Sci.*, **15**, 163; 1974 and unpublished).

As an alternative explanation for their findings Sutherland *et al.* (1975) suggest that the mutations in XP affect a regulatory gene common to photoreactivation and dark repair, but this explanation too is difficult to reconcile with the complementation data. May one wonder, then, whether the proteins defective in XP act as or, perhaps more likely, favour the function of the photoreactivating enzyme? Might many of the enzymes of the DNA repair systems be cointegrated to form a complex whose diverse functions could be differentially upset by mutations in different component proteins?

In spite of present uncertainties, XP clearly demonstrates the importance of DNA repair and its relevance to oncology. It offers an opportunity to study the genetics, physiology and biochemistry of DNA repair in man and, in addition, by drawing our attention to photoreactivation and to different dark repair pathways, it sets us the task of determining the relative contribution of these processes to cellular genetic homeostasis.

Much still needs to be done to clarify the genotype–phenotype relationships in XP. Of particular interest are the neurological abnormalities, which seem to result from diffuse and premature neurone loss. This process may provide clues to understand the ageing of non-regenerating tissues and organs such as the brain. Does, for example, the absence of neurological abnormalities observed so far in XP-variants, suggest that excision repair is of primary importance for non-regenerating tissues and consequently that DNA damage has a key role in ageing (see Burnet, *Intrinsic Mutagenesis: A Genetic Approach to Ageing;* Medical and Technical, Lancaster, 1974)?

The lithosphere as prime mover

from Peter J. Smith

ALTHOUGH the broad tenets of plate tectonics are now widely understood and accepted, there is still ample scope for the investigation of, and disagreement over, the all-important details. One of the most crucial of these 'details' is the precise mechanism which allows continents to drift, seafloors to spread and hence plates to interact. When the geophysicists of the 1940s and 1950s finally broke the chain of disbelief in Wegenerian drift by proposing the existence of convection currents in the Earth's mantle, they had in mind a system in which the relevant parts of the overlying crust or lithosphere were carried along as the passive partners of a self-contained driving force below. This idea was then later modified to enable the lithosphere to become an integral, albeit still passive, component of the convection pattern. More recently, however, some workers have suggested that the lithosphere is not only a part of the convective cycle but the active part. According to this view, either the cold lithosphere descending at subduction zones pulls the plate or the hot lithosphere created at ridges pushes it—and the asthenosphere merely acts as a counterbalancing channel for the flow of material in the opposite direction.

Which, if any, of these mechanisms actually operate (they are not mutually exclusive) is anyone's guess at present; but in an attempt to reduce the options Harper (*Geophys. J.*, **40**, 465; 1975) has constructed a model which enables certain plate parameters to be predicted and hence compared with observed values. Harper's basic premise is that, irrespective of what the principal driving mechanism is, subduction zone pull and oceanic ridge push must play some part. His model then assumes that these are the only driving forces and that lithospheric motion is resisted only by Newtonian viscous drag in an asthenosphere in which the return flow is everywhere antiparallel to the plate velocity.

The first prediction of the model is that, if the commonly accepted ('reasonable') values of density, viscosity and plate thickness are correct, a typical subduction zone pulls about seven times as hard as a typical oceanic ridge pushes. This is not a conclusion which can be tested directly, of course; but it is an interesting one nonetheless, especially as it is likely to confirm many an intuitive belief based simply on the relative scales of subduction zones and oceanic ridges.

More importantly perhaps, Harper's model permits estimation of the angular velocities of plates, from which relative angular velocities between plate pairs may be calculated. As far as pairs among the Antarctic, Pacific, Indian, African, American, Eurasian, Nazca and Cocos plates are concerned the agreement between the relative angular velocities predicted from the model and those observed—as quoted by Chase (*Geophys. J.*, **29**, 117; 1972)—is regarded by Harper as no more than 'tolerable' in most cases, and for the Cocos plate in particular rather bad. The Cocos example is instructive, however, in that it leads to a refinement of the original model which not only brings the Cocos plate into line with the others but improves the agreement between prediction and observation in all cases. There are several possible problems with the Cocos plate, one of the most obvious being that it is small. But it is also relatively thin. When the relative thicknesses and the thickening with age of all plates are taken into account, predicted relative angular velocities for the various plate pairs range from 0.81 to 1.38 of the observed velocities (compared with 0.80 to 8.8 for the unmodified model and 1.0 for perfect agreement).

Considering the simplicity of the model (even the modified model), the approximations incorporated into it and the uncertainties in the numerical values adopted, the agreement between prediction and observation is really quite remarkable. Indeed, Harper himself seems somewhat surprised, arguing that coincidence cannot be entirely excluded. But taken at face value, the results suggest that the motions of the eight principal plates can be largely accounted for without considering any driving forces but the downward pull at subduction zones (especially) and the outward push from oceanic ridges.

In addition, as a result of considering the stresses acting within tectonic plates, Harper is able to throw some possible light on to the vexed question of why and how the ancient supercontinents split apart in the first place. Prior to the opening of the Red Sea, the eastern boundary of the then African–Arabian plate would have had a re-entrant corner to the east of the tip of what is now the Somali Republic. According to Harper such a corner would magnify the (tensile) stresses locally, facilitating the formation of a crack. Certainly a crack developed in this case, propagating towards the southern tip of Arabia and then, with a change of direction, up what was to become the Red Sea (a course presumably dictated partly by local geology and partly by the forces pulling towards the subduction zone beneath the Zagros mountains in the north-east). But this change of direction would have produced a new re-entrant corner at the southern tip of Arabia, a new local concentration of stress and (as evidently occurred) a new split forming the east African rift.

Would it be possible to explain the gradual break up of Gondwanaland (or even Pangaea) entirely in terms of a series of cracks propagating from re-entrant boundary corners, perhaps aided in many cases (once the process got going) by pulling at appropriately situated subduction zones?

Faithful transcription *in vitro*

from T. Barrett

RECENTLY much interest has focused on reproducing *in vitro* the transcriptional ability of native chromatin. The manipulation of chromatin during its isolation, most notably shearing to render it soluble, is liable to alter its template activity, and it is reasonable to be sceptical about the relationship that *in vitro* transcription products bear to RNA synthesised *in vivo*. De-Pomerai *et al.* (*Eur. J. Biochem.*, **46**, 461; 1974) demonstrated that chromatin prepared by various published methods differed both in structure, as defined by the DNA : RNA : protein ratio, in template activity using both endogenous and added homologous RNA polymerase, and in the size of transcript produced. Shearing of chromatin resulted in a decrease in transcript size and an altered size distribution on formamide sucrose gradients. These authors suggested that extraction of chromatin and RNA polymerase from eukaryotic cells may remove factors necessary for *in vivo* transcription and subsequent processing of RNA.

More recently Noll *et al.* (*Science*, **187**, 1203; 1975) demonstrated that the structure of native chromatin is easily destroyed when chromatin is prepared by conventional methods involving shear. Such chromatin does not have the repeating structure shown by native chromatin when digested by micrococcal nuclease. They have developed a technique whereby the native structure is retained, which involves slight nuclease digestion of isolated nuclei followed by lysis in hypotonic buffer. The resulting chromatin shows the same 200 base pair repeating unit found in nuclear chromatin. But they point out that chromatin, even when prepared by this comparatively gentle method, is not necessarily identical with chromatin in the nucleus.

The most direct approach to the study of fidelity of transcription *in vitro* has been to search for specific RNA sequences in the transcription product. Paul and Gilmour (*J. molec. Biol.*, **34**, 305; 1968) first indicated that organ-specificity was retained in isolated chromatin, but their hybridisation techniques only provided information about RNA transcribed from reiterated DNA sequences. The use of reverse transcriptase to copy specific messenger RNAs into highly radioactive complementary DNAs has, however, made it possible for Axel *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2029, 1973) and Gilmour and Paul (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 3440; 1973) to demonstrate that RNA transcripts of chromatin isolated from erythropoietic tissues contain globin-specific RNA sequences.

These studies were performed using bacterial RNA polymerase and indicated nothing about the accuracy of transcription of the globin genes on isolated chromatin. Steggles *et al* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1219; 1974) compared the transcription of globin-specific RNA sequences on rabbit bone marrow chromatin using both bacterial and mammalian form B RNA polymerase. Both enzymes transcribed globin-specific RNA sequences, but with mammalian enzyme globin sequences represented a higher proportion of the RNA synthesised (0.05% for mammalian as opposed to 0.016% for the bacterial enzyme). The use of homologous rather than bacterial polymerase, however, does not provide the complete answer to the problem of accurate *in vitro* transcription. Honjo and Reeder (*Biochemistry*, **13**, 1896; 1974) found that added homologous RNA polymerase transcribed the rRNA genes of *Xenopus laevis* chromatin in an aberrant manner. The polymerase transcribed both strands and the spacer regions and in fact was no more accurate than bacterial polymerase in this respect.

In a recent paper Marzluff and Huang (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1082; 1975) claim to have achieved faithful transcription of low molecular weight RNA species using the endogenous RNA polymerase of mouse myeloma chromatin. Chromatin was isolated from nuclei by lysis in hypotonic buffer and then sheared by passing it through a narrow gauge syringe needle. The accuracy of transcription of the 5S RNA and tRNA genes *in vitro* was confirmed by the correct size of the transcripts, 5S for the 5S gene and 4.5S for the precursor of 4S tRNA. In addition correct strand transcription was indicated for the 5S RNA gene since the transcript hybridised only to the + strand of *Xenopus laevis* 5S DNA. Although Zylber and Penman (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2861; 1972) and Reeder and Roeder (*J. molec. Biol.*, **67**, 433; 1972) had previously shown that rRNA is faithfully transcribed in isolated nuclei, it is of interest that in isolated chromatin where half the nuclear protein and 90% of the nuclear RNA is removed, an accurate transcript of the 5S and 4S genes can still be obtained. There are differences, however, between isolated nuclei and isolated chromatin. Marzluff and Huang found that the chromatin system was much less stable as regards RNA synthetic activity. Activity is rapidly lost at 4 °C and is completely destroyed by freezing. The average molecular weight of the total RNA transcript from chromatin is lower, probably because of the shearing process employed during chromatin preparation, although this does not appear to affect the low molecular weight RNAs which are still synthesised accurately. In chromatin no 4S RNA was identified, although a 4.5S precursor species was synthesised. This contrasts with the nuclei where some 4S RNA is produced and suggests that the enzyme responsible for cleaving the 4.5S precursor is missing or inactive in the chromatin preparation.

In spite of the differences, the preferential incorporation of γ-^{32}P-GTP into 5S and 4.5S RNA suggests that these species are being reinitiated and transcribed continually *in vitro*. The genes for 5S and 4S RNA represent only a small portion of the nuclear DNA (0.001% and 0.005% respectively) but they account for nearly 1,000 times that amount of RNA, indicating that strict template restriction is maintained *in vitro*. Marzluff and Huang suggest that the structure of these genes in chromatin and their interaction with factors other than polymerases may result in their great activity *in vitro*. This may explain why isolated polymerases, whether homologous or bacterial, do not show the same extensive restriction when used to transcribe chromatin. Such studies emphasise that the native structure of chromatin and its intimate association with some, as yet unknown, labile factors may be absolutely essential for the accurate transcription of genes *in vitro*.

review article

Non-Darwinian "evolution" and biological progress

J. M. Thoday*

The use of terms such as "higher" and "lower" to describe different forms of life shows that we tend to think of evolution not only as adaptive, but also in some sense as progressive. That sense can be defined in a way that makes it possible to show how some "neutral" polymorphisms could contribute to progressive evolution.

PROGRESS and its adjective progressive are ambiguous words. They may imply merely unidirectional change, or hey may imply change in a direction that is regarded as mprovement. They have been used in both senses (someimes both senses are confounded) in writings about evoluion, but though there have been difficulties in defining progressive evolutionary change in the sense of improvement, many authors have clearly felt that evolutionary changes have given rise to improvement. Indeed most feel so: otherwise the use of higher and lower as applied to various animal and plant groups would be seen to be empty of meaning.

Some time ago[1,2], I made an attempt to define "biological progress" in a way that I thought avoided the circularity of the arguments that had been used by Julian Huxley[3,4] whose definition of progress was based on the implicit assumption that progress has in fact occurred. Stebbins' recent book[5] also makes this implicit, and it seems to me, unwarrantable assumption.

My attempt avoided this assumption by defining progress solely in terms of the general conditions of survival. Most of the trends Huxley and Stebbins identified as progressive all into progress as I defined it either under the headings of phenotypic flexibility (see below) or environmental control.

Current controversy about the role of neutral alleles in volution prompts me to consider to what extent such neutral alleles may contribute to progressive evolution as o defined.

Neutral alleles and adaptive evolution

The controversy concerning neutral alleles centres on the question of the proportions of genetic differences produced by mutation that can be regarded as selectively neutral, nd on the extent to which such spread of neutral alleles may explain differences between populations and species nd also explain the number of alleles at a locus to be found in a population. Since the spread of neutral alleles s a purely chance process, such evolution has been called non-Darwinian[6].

The theory of evolution has two major components, the oncept of evolution itself and the mechanism of change that has brought evolution about.

The concept of evolution explains the classifiability of organisms, the facts of plant and animal geography, the common behavioural, morphological, embryological, anatomical, physiological, biochemical, cytological and genetical properties of diverse organisms, the facts of palaeontology and palaeobotany, results of the study of microevolution in "nature" and in the laboratory, and the results of plant and animal breeders. It does so by postulating that diversity is the consequence of modification over the generations of differing lines descended from common ancestors. It does not explain adaptations as such but explains the diversity of adaptations.

Our concepts of the mechanism of evolution seek to explain the change that gave rise to this diversity of adapted organisms, and here it is essential that an evoluary theory must explain adaptedness and changes of adaptive mode. Natural selection is the agent central to Darwinian evolution because it explains adaptedness. Modern Darwinian theory has the following components: mutation, random with respect to place, time and need, which provides the source of allelic heterogeneity that is the basis of change; segregation and recombination that provide varying combinations of alleles and diversity of genotypes; selection which increases the relative frequency of the genotypes that on the average lead to the fitter (relatively better adapted) phenotypes; and genetic drift which leads to random changes in allele frequency and in small populations may establish an allele in a population sometimes even if that allele leads to phenotypes less fit than some alternative allele and which will certainly establish neutral alleles. Those of strong selectionist views would allow only a very minor role for drift, except in its special forms of "founder principle" and when there are very restricted bottlenecks in population size.

Non-Darwinian evolution, on the other hand, would give drift a major role in molecular evolution by postulating that a large proportion mutations leads to neutral alleles, that is to say to alleles that do not differ in their effect on fitness from the alleles that mutated.

Two interesting points emerge. One is that these new developments would greatly increase the role of chance in the determination of the direction of evolution. Darwinian evolution involves considerable elements of chance, not only with respect to drift, but also to the timing and direction of mutation and the chance involved in the origin of particular combinations of genes. It is of interest here to draw attention to the fact that it is this chance component of evolutionary theory to which many (see for example ref. 7) object, with its consequences that the precise course of evolution is unpredictable, that evolution could have

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taken many other paths, that any particular species or individual must be regarded as an accident, and that evolutionary changes are not necessarily either adaptive or progressive; these are aspects of the theory that do not conform with any of our fundamental philosophies or with our egocentric outlook. Non-Darwinian evolution would simply add a greater component of chance.

The second point is that in so far as substitution of neutral genes by chance processes is regarded as "evolution" it must be questioned whether the "evolution" it considers is the same as "evolution" considered in the Darwinian sense.

Population geneticists have come to talk of changes of allele frequencies as evolution. But evolution, in the classic sense, involves adaptive changes of phenotype. Neutral allele substitutions cannot be adaptive, and, from the point of view of adaptive evolution theory, it seems doubtful whether a genotypic difference which can never make any difference to fitness is to be regarded as relevant. The relevance to adaptive evolution of a genotypic difference which makes a difference that we can detect at some level we call phenotype, but which makes no difference to the economy of the organisms, is questionable.

In discussing whether "neutral" alleles might in any sense be relevant to adaptive evolution we must therefore consider the kinds of neutral allele pairs we may envisage. There are four, of which we shall see that only two, conditionally neutral and pseudo-neutral allele pairs, can be envisaged as playing any role in adaptive evolution.

Strictly neutral allele pairs

These are pairs of alleles that can in no circumstances make any relative difference to fitness and therefore can in no circumstances contribute to adaptive or progressive evolution. They may include redundant codon differences, though it should not be forgotten that these might differ in efficiency of transcription or replication, and that some redundant codon pairs may involve some recombinational load, for example UUA and CUU both code for leucine but can recombine to give UUU phenylalanine.

They may also include allele pairs that are meaningful codon differences but lead to "redundant" amino acid substitutions in parts of a protein that do not affect the protein's function. Some of these differences may be detectable by techniques other than sequencing, for example tests of electrophoretic mobility, heat stability, enzyme activity, and thus have some detectable effect on some aspects of phenotype, but might nevertheless have no effect on fitness. It seems fair to suggest that the more readily detectable at such levels the differences are, the more likely they are to affect fitness.

Among these differences there may be some that have an overt effect on gross phenotype. The history of population genetics, in which one after the other overt polymorphism has been claimed to be neutral but has subsequently been proved to be maintained by selection, should lead to some scepticism concerning this possibility.

Quasi-neutral allele pairs

These have been proposed by Kimura, and the proposition might be regarded as a retreat from the more strictly neutralist position though he originally[8] did refer to "neutral or nearly neutral" alleles. They are alleles that affect fitness, but do so to such a small extent that their fate can be accurately predicted by neutral theory. Unless, however, their negligible effect on fitness can change (in which case they are conditionally neutral, see below) they are as unlikely to be relevant as strictly neutral alleles.

Conditionally neutral allele pairs

These are allele pairs that were neutral or quasi-neutral in past circumstances but may be non-neutral in some future genetic or external environmental circumstance. To separate these as a category is important for they may be envisaged as a class of neutral alleles that may be established and maintained in a population without affecting fitness, but in some other circumstances might form a source of genetic variance that through selection could improve adaptedness.

As an illustrative model example we might envisage a pair of alleles differing in the heat stability of the enzyme they code for: say that one enzyme is inactivated at 33 °C, the other at 40 °C. Assuming that, below the critical temperature 33 °C, the isoenzymes have the same biochemical properties, and also assuming that the environment of the population never involves temperatures higher than this, the alleles will be neutral. But if the environment changes, or if some part of the population migrates to some new area, by accident or as a result of change elsewhere in the genome that enables the population to spread out of its hitherto restricted range, so that temperatures above 33 °C are now met, then the alleles cease to be neutral and natural selection will begin to affect their frequencies. The conditionally neutral polymorphism might then be said t be preadaptive.

Pseudo-neutral allele pairs

These are of two kinds. The first are similar to conditionall neutral alleles. They are those which were in the past non neutral but, because conditions have changed, are neutra now. They must of course contribute to the pool of conditionally neutral alleles now since the change of condition might be reversed, but they must be distinguished becaus their presence in the population is a result of selection s that they, being neutral now, will contribute to the diffi culty of determining what proportion of molecular poly morphism results from non-Darwinian evolution.

The second are alleles which are neutral or quasi-neutra in present circumstances because selection has made the so. Among these we include those showing frequenc dependence, for by definition these at equilibrium ar neutral, but their neutrality is itself a consequence of selec tion. Cyclic selection, niche-dependent disruptive selection especially with habitat choice[9], and density dependent selec tion may all contribute to such pseudo-neutrality. Balance polygenic systems as envisaged by Mather would also in volve pseudo-neutrality, the relative fitness effects of allele at the separate loci being neutralisd by their balancing wit alleles of opposite effects in linked loci. Modifiers may fa into this category also.

There is good evidence that some molecular difference are subject to such frequency dependent selection. (Se Kojima and Yarbrough[10] for direct frequency dependence Powell[11] or Ayala[12] for niche-dependent disruptive sele tion.) Furthermore we have no reason to suppose that man of the molecular differences we can detect by such tec niques as electrophoresis are not part of polygenic system just as may be differences in reiterated DNA.

Pseudo-neutral genes are clearly subject to Darwinia evolution.

Neutral alleles and progressive evolution

In considering the possible contribution of neutral allel to progressive evolution we must have in mind the chang that fall under the definition of progressive changes.

To survive, in anything but the short term, a popul tion or species must have in sufficient measure each three properties, adaptedness, stability and variability.

These three concepts express the three basic conditions of continued life, and two of them, genetic stability and variability, are in part antithetic, for they express the need to maintain present adaptedness through stable heredity, but at the same time to preserve adaptability to future different environments.

It needs to be stressed first that we are by no means solely concerned with inorganic sources of environmental change, for evolutionary change of one species is an important environmental change for others that interact with it. Second that, at least from the point of view of the organisms, future environmental change, and hence the adaptation that will be required, is unpredictable. Adaptation to the unpredicatable requires the generation of variance at random[13], but to generate variance at random necessarily involves a reduction of genetic stability. We can therefore see that progressive evolution must be evolution that leads either to a reduction in the need to generate random variance, or to the storage of such variance in temporarily ineffective forms.

In my original discussions of this concept of progress[1,2], I pointed to various ways in which these ends have been achieved. These can be summarised under the headings of the various components of long term fitness as follows.

(1) Adaptedness: this expresses the degree to which individuals fit contemporary environments, and within populations relative adaptedness is equivalent to Darwinian fitness. When we are comparing species or major groups, however, since most of them survive well in their contemporary environments, we cannot look to adaptedness in this sense when looking for evidence of progressive evolution.

(2) Genetic stability: this expresses the capacity of individuals to produce adapted offspring, and is promoted by asexual reproduction, inbreeding, homozygosity, and low mutation rates.

(3) Variability: this expresses the capacity of a population to change and depends on (*a*) genetic flexibility, which is the capacity of the population to produce offspring different from their parents, and is promoted by high mutation rates, heterozygosity and outbreeding; and (*b*) phenotypic flexibility. Phenotypic flexibility expresses the range of environments in which the individual can function, or the ability of the individual to "recognise" and move from unfavourable to favourable environments. It is this component of fitness that covers most of the progressive trends recognised by Huxley[3] as making organisms relatively independent of the environment, or rather of certain aspects of environmental variation. Some would include phenotypic flexibility as part of adaptedness, but I separate them because such flexibility is part of adaptability. Recent developments show that relatively "simple" organisms have more phenotypic flexibility than might hitherto have been thought for the gene control systems of, for example, *E. coli*[14] or *Aspergillus nidulans*[15] provide efficient biochemical flexibility allowing the individual to adapt to variation in the external environment.

(4) Stability of environment: this determines the need for change which can be reduced by (*a*) specialisation for stable components of environment, a risky process because the continued stability of the habitat is unpredictable, and (*b*) control of environment directed towards maintaining its suitability, or making it more suitable and keeping it so.

It is the changes that may lead to partial resolution of this antithesis between the requirements of stability and variability that we must define as progressive changes. The antithesis lies between genetic stability and genetic flexibility and is resolved by any increase of phenotypic flexibility, by the adoption of a stable habitat, or of environmental control (which requires the development of ommunities and, in the ultimate, ability to predict in order to correct environmental trends).

These are ways that reduce the need for genetic flexibility. But the direct antithesis between genetic stability and genetic flexibility has also been reduced by two developments, and may possibly have been reduced by a third.

The first is through the adoption of dual breeding systems, asexual reproduction providing stability and sexual reproduction providing flexibility.

The second is through the development of genetic systems that permit the storage of potential variation[16,17] without consequent variation of fitness. Such storage is achieved through dominance in diploids, the heterozygous recessives being a source of variance that is "hidden". In diploids and haploids, balanced polygenic systems provide a source of stored potential variation. In outbreeding populations the fixed variation is freed at a rate determined largely by recombination. In inbreeders, complementary alleles may be fixed in different more or less homozygous lines, and the freeing of this variation depends on the frequency of hybridisation (outbreeding) between such lines and on the subsequent recombination in the hybrids. Recombination frequencies are of course also under genetic control, which may perhaps be relaxed in unfavourable circumstances, after wide outcrossing, or as a result of intensive selection, so that the rate of release of potential variation may itself be increased when genetic flexibility is most needed.

The third possibility is that in unfavourable circumstances the control of replication, including DNA repair systems, may be impaired, thus leading to increase in mutation rate when genetic flexibility is most needed[18].

It is clearly in respect to the resolution of this antithesis between genetic stability and genetic flexibility, that neutral allele polymorphisms might be important.

Strictly neutral allele pairs clearly make no contribution to short term or long term fitness, and if they exist they are mere evolutionary noise.

On the other hand those conditionally neutral allele pairs that have been established in populations by non-Darwinian evolution, but may in some future conditions cease to be neutral, provide in principle a potential source of genetic flexibility that does not involve loss of genetic stability. They thus provide an additional source of stored potential variation. Polymorphisms for such conditionally neutral alleles may therefore provide a source of adaptation to the unpredictability of the environment. If so, non-Darwinian evolution is an additional source of evolutionary progress.

I conclude that neutral allele pairs can only contribute to evolution in the classic sense, as distinct from evolution defined as mere gene frequency change, if the neutrality is "conditional". The establishment of unconditionally neutral alleles is mere evolutionary noise. The establishment by "non-Darwinian evolution" of polymorphisms for neutral alleles which may in future combinations or conditions cease to be neutral may provide an important source of genetic flexibility that does not impair genetic stability. The establishment of such polymorphisms would be an additional form of progressive evolution.

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articles

An electrical conductivity anomaly and rifting in southern Africa

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Measurements of electrical conductivity provide information about rifting in eastern and southern Africa. Overall geophysical and geological evidence supports the view that the conductive zone marks an extension of the African Rift system along old weak zones in the lithosphere.

A MAGNETOMETER array study in 1972 (J. H. de B. *et al.*, unpublished) discovered a zone of concentrated induced electric currents at lithospheric depths crossing South-west Africa, Botswana and western Rhodesia. The array of 25 three-component magnetometers gave results from which three periods of magnetic disturbance were analysed. Magnetograms and maps of Fourier transform amplitudes and phases show a large anomaly in the vertical and northward horizontal variation fields over the period range 21–171 min. A well defined reversal in the vertical field is observed in South-west Africa between 20°S and 21°S. Transfer functions between mean horizontal field components and the vertical component show a conductor that trends east–west in the western part of the array and curves north-eastward in the eastern part. The half-widths of normalised anomalous fields and the induction vectors indicate that the conductor is at crustal depth. In South-west Africa the conductor is well covered by the array and is no more than 100 km wide (Fig. 1). In Botswana the conductive zone is wider and its limits are less adequately defined by the array. A south-westward continuation is possible. At the north-east limit of the array its maximum width is about 250 km (Fig. 1). From east to west the conductive zone continues the south-westward line of the Luangwa–middle Zambezi Rift (Fig. 2) and bends south of the Okavango Delta to run nearly westward across South-west Africa (Fig. 1).

To test the hypothesis that the conductive zone indicates a continuation of the known rift system of Africa, we note other relevant evidences of such a continuation. In north-eastern South-west Africa and all of Botswana the Kalahari sands of Tertiary or younger age cover the hard rocks and hide almost all tectonic features[1]. Boreholes reveal Mesozoic Karroo sediments or volcanics under much of the Kalahari basin[2] (Fig. 1) but have not so far penetrated the recent sediments in the Okavango Swamps (D. Hutchins, personal communication). Major linear features with a north-east trend are found near the Okavango and have been interpreted as faults[2,3]; they continue the line of the middle Zambezi–Luangwa Rift. The middle Zambezi valley, containing Lake Kariba, is an asymmetric rift preserving Karroo rocks downfaulted between Precambrian shield rocks; its fault pattern is reasonably well known[4,5]. Details of the Luangwa Rift faults are lacking[6] but the valley floor is largely covered by Karroo sediments and several igneous centres are known[4], including Cretaceous carbonatite volcanoes[6,7]. Carbonatites and kimberlites occur also in the middle Zambezi[8] and kimberlites near Orapa in Botswana (Fig. 1). As early as 1921 Gregory[9] considered the Luangwa–middle Zambezi Rift as an extension of the East African rift systems better developed and known in Kenya and Ethiopia. Others[7,10] have already used geological arguments to propose south-westward continuation of the Luangwa–middle Zambezi Rift into Botswana.

Seismological evidence

Considerable seismic activity with magnitudes up to 6.7 occurred in the Okavango Delta region in the period 1949–55 with 90% of events in late 1952–early 1953 (refs 11 and 12) Reeves[2] mapped 50 earthquakes in Botswana over the period 1965–71, and found a concentration of epicentres in the Okavango Delta and scattered events in the central Kalahari, with the broad Ghanzi Ridge of post-Damara sediments between the two seismic regions (Fig. 1). In the Lake Kariba region a low initial level of seismicity rose several orders of magnitude as the lake filled, culminating in three events of magnitudes near 6 in 1963 just after the lake level peaked. Triggering of renewed activity on the existing faults, by incremental solid stress rather than water pressure, was suggested by Gough and Gough[5] Since 1963 activity has continued with very slow if any decline, in a manner consistent with the hypothesis tha the region is now seismic in a steady state. Mechanisms fo two of the large events show normal faulting with on nodal plane striking N25°E parallel to regional faults[13,14] These and other fault plane solutions for earthquakes i eastern and southern Africa[13,15] suggest tensile deviatori stress in a WNW–ESE direction. Fairhead and Girdler showed that in the period 1963–70 earthquakes in souther central Africa were located in a broad seismic zone runnin southward from Lake Tanganyika to a point just west c Lake Kariba Except in the Lake Kariba area the middl Zambezi–Luangwa Rift was virtually aseismic throug 1963–70.

Epicentres from several sources (refs 16–18 and L. M Fernandez and J. A. Guzman, unpublished) for the perio

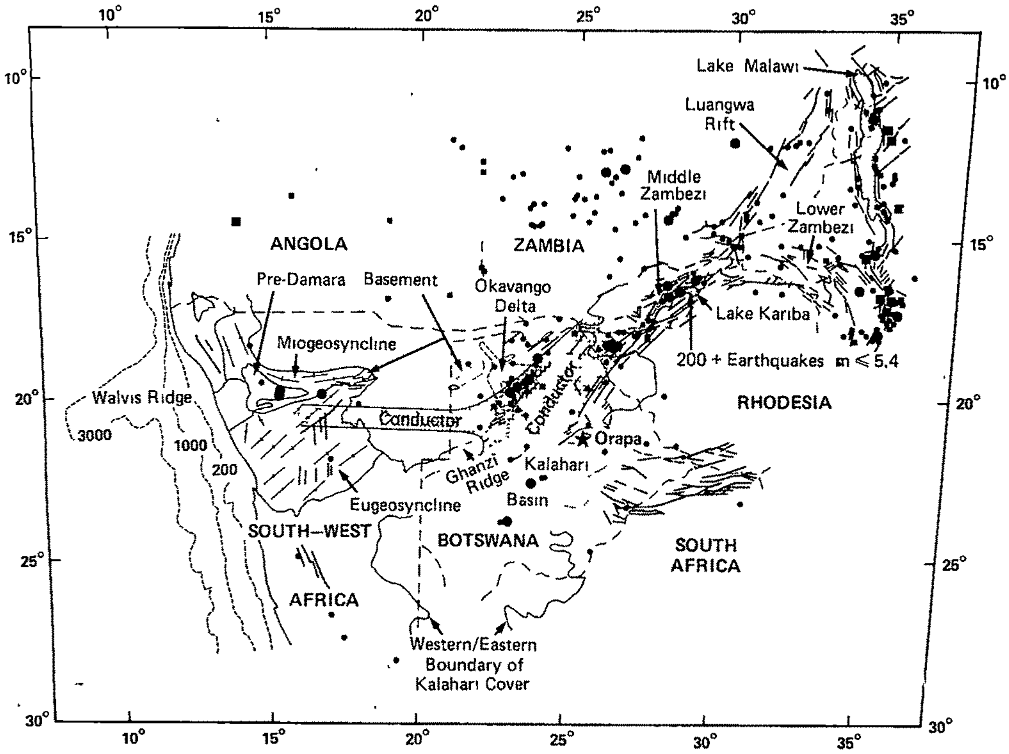

Fig. 1 The conductor in relation to seismicity and tectonics of southern central Africa. Small dots, $2 \leqslant m \leqslant 4$; large dots, $m > 4$. Small squares, intensities < 3; large squares > 3. Faults (bold lines) are from refs 3, 6, 40–42. Fold axes (with crossbars) are from ref. 1. Geological boundaries are from refs 1, 3 and 40. Offshore depths are in metres.

1965–73 have been plotted in Fig. 1. Their distribution supports the hypothesis that the Luangwa–middle Zambezi Rift continues to the south-west into Botswana. Focal depths range up to 50 km (refs 17 and 18).

Westward extension

West of the Okavango the conductive zone runs a few degrees north of due west to the exposed Damara Geosyncline of South-west Africa (Fig. 1), where it cuts obliquely across the WSW–ENE grain[1,19] of metamorphosed rocks of the eugeosynclinal Swakop Facies of Upper Proterozoic–Lower Palaeozoic ages[19]. To the north of the Swakop Facies, the miogeosynclinal Outjo Facies has E–W strikes in its eastern exposures. The conductor runs roughly parallel to the folding in eastern outcrops of Outjo rocks, and to the boundary between the eugeosyncline and the miogeosyncline. It also parallels the northern limit of Damara regional metamorphism[19]. No recent major faults are known to run parallel to it. An association with the boundary between two stable crustal blocks seems possible. Several earthquake epicentres lie close to the northern edge of the conductor (Fig. 1).

Origin of conductive zone

Regions of high conductivity which produce geomagnetic induction anomalies may be related to high temperatures in the upper mantle, as in the western USA[20], or to compositional conductors such as graphite or saline water which are often concentrated in fracture zones[21,22]. If the conductive zone under discussion is associated with the African rift system its conductivity could be associated with anomalously high temperature in the upper mantle, or with a fracture zone in the lithosphere, or with both. No decision is possible, but we believe a fracture zone is the likeliest explanation. From Kariba to the Okavango, fractures parallel to the conductor are visible and seismically active (Fig. 1). Seismological evidence for the mantle under the East African rifts shows low P velocities and attenuation of S_n north of a discontinuity near latitude 4°S (refs 13, 15 and 23). For a path Addis Ababa–Nairobi, Rayleigh wave phase velocities are low for a continental path[24] and resemble those in the Basin and Range Province of western North America. Elsewhere in Africa phase and group velocities of surface waves and travel times of P_n, S_n and L_g are typical of stable continental regions[25,26]. The eastern (Gregory) rift shows a broad negative gravity anomaly north of 4°S and a central positive anomaly, usually interpreted as evidence of intrusion of mantle-derived material, north of 2°S (refs 27–31). There is no central positive anomaly and thus no evidence of a central intrusion under the western rift[15]. For no rift is there geophysical evidence of partial melting in the mantle south of 4°S, at which latitude the fracture pattern in the eastern rift changes from grid faulting in the north to block faulting in the south[32]. Geomagnetic depth sounding across the eastern rift at the Equator[33] reveals anomalies accountable in terms of a conductive region 20 km below the rift floor and another 50 km deep and 100 km east of the rift. Soundings south of 5°S might give different results. Gravity data south of 5°S are sparse and ambiguous and a recent unpublished Bouguer anomaly map of Botswana[43] has both highs and lows along the conductor.

The north-east trending arm of the conductor (Fig. 1) can reasonably be associated with the known fractures, whose activity resembles that of the western, Lake Tanganyika rift. The absence of known fractures along the westward arm of the conductor does not preclude their existence because of the Kalahari cover, and seismic activity is present. The westward arm is in line with the Walvis Ridge (Fig. 1), which may, on recent geophysical evidence[34], be a fracture zone, either a transform fault or some tectonic process following an older line of weakness; the conductivity anomaly could then lie on a corresponding line of weakness within the continent, along the boundary in pre-Damara basement rocks between the eugeosyncline and the miogeosyncline. In southern Africa pre-existing cratons and orogenic belts have often controlled later tectonics[4,35–37]. The seismicity may thus trace contemporary failure, under a general WNW–ESE tensile stress field, on pre-existing fractures. The first down-faulting in the middle Zambezi probably occurred during early Mesozoic

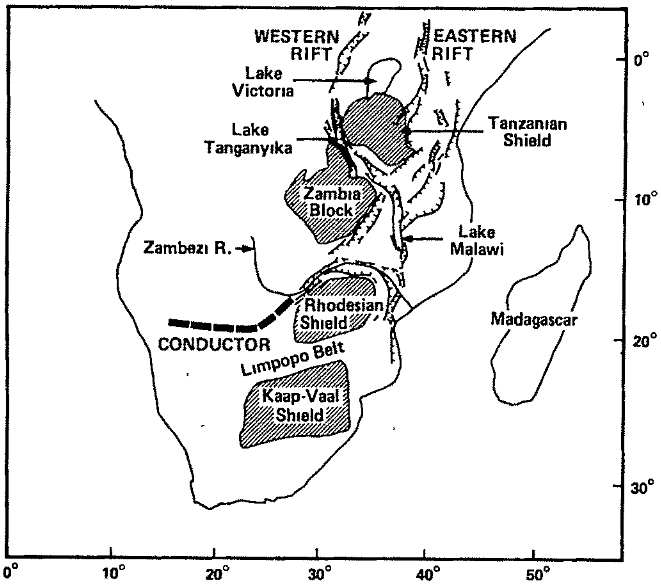

Fig. 2 African rifts and shields in relation to the conductivity anomaly (compiled from a map by McConnell[35], and other sources).

times[4,5] along fractures in the Upper Proterozoic-Lower Palaeozoic mobile zone related to the tectono-thermal Katangan and Damaran Episodes[19]. The conductor could mark this zone of weakness in the lithosphere[38] along which intermittent movement has occurred since the Upper Proterozoic.

The absence of evidence for opening of rifts in southern Africa can be related to global tectonics in two alternative ways. In terms of classical plate tectonics the pole of opening for the East African rift is in southern Africa[13,23], where opening will therefore be small. On the membrane stress hypothesis of Oxburgh and Turcotte[39] the rifts are caused by northward movement of the continent over the equatorial bulge. Again propagating fractures with small displacements would be found in southern Africa.

Received April 15; accepted May 21, 1975.

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New method for mapping genes in human chromosomes

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If human cells are subjected to large doses of ionising radiation and then fused with rodent cells, hybrid clones are obtained in which linked human genes may be segregated. By measuring the frequency with which pairs of linked genes are cotransferred after irradiation, it is possible to determine the linear order of groups of genes and to estimate the distances between them. This technique offers a new and systematic approach to the mapping problem.

RECENT progress in the assignment of genes to specific human chromosomes has been made possible by four developments: first, the provision of a general method for fusing together cells from different animal species and the demonstration that such fused cells can give rise to mononucleate hybrids capable of indefinite multiplication[1,2]; second, the application of selective media to facilitate the isolation of the hybrid cells[3]; third, the discovery that in hybrids produced by fusing human cells with those of rodents most of the human chromosomes are rapidly and preferentially eliminated[4]; and finally, the detection, in appropriately stained preparations, of specific banding patterns in the chromosomes that permit their accurate identification[5] By means of experiments in which the retention of human biochemical markers in man–mouse (or man–hamster) hybr cells is correlated with the retention of identifiable human chromosomes, some fifty human genes have been reliably assigned to specific chromosomes[6]. The success of these experiments does not, however, alter the fact that, compared with what can be achieved in prokaryotic systems, this form of linkag analysis, limited in whole chromosomes, is a very crude form of mapping. Some modest success in determining the positio of genes within the chromosome has been achieved by exploiting translocations that segregate linked markers[7-9]. But this approach is unsystematic in that it is constrained by the availability of informative translocations involving the particular chromosome being studied, and limited to resolution in that genes can only be localised to a segment of chromosome large enough to be identified in a translocation. For mammalian cells, a general method for mapping the order and spacing of linked genes is still lacking.

In this paper we present a new approach to the mapping problem and illustrate its efficacy in determining the position of four genes on the long arm of the X chromosome in man. The principle underlying this approach was suggested by two sets of observations. First, chromosomes may be fragmented and parts of chromosomes translocated by ionising radiation; the frequency of chromosomal rearrangements is reproducibly determined by the dose of radiation administered[10]. It will be obvious that when chromosomes are fragmented, the probability of two linked genes being included within a single fragment will be inversely related to the distance between them. Second, fragments of foreign genetic material can, in certain conditions, be introduced into mammalian cells in such a way that they are replicated and expressed even though they may be too small to be detectable in cytological preparations[11-14].

We therefore subjected human cells to controlled doses of radiation, fused them with unirradiated hamster cells and selected clones of hybrid cells expressing a marker known to be coded by a gene located on the long arm of the human X chromosome. We then measured the frequency with which three other genes known to be located on this arm of the X chromosome were cotransferred with the selected marker. From these measurements we were able to determine the order of the four genes and estimate the relative distances between them.

Selection of hybrid clones

Human diploid male lymphocytes, purified from venous blood by the Ficoll–Isopaque technique[15], were irradiated and fused immediately after irradiation with the hamster cells. The lymphocytes, which had not been subjected to any specific mitogenic stimulation, were thus very largely a non-dividing cell population; each cell contained only one copy of the X chromosome. The same lymphocyte donor was used for all experiments to reduce as far as possible variation between different batches of cells. The conditions of irradiation were made as reproducible as possible: the equipment embodied a double caesium source which delivered gamma rays at a constant dose of 120 rad min^{-1}. During irradiation cells were suspended in ice-cold Eagle's MEM without serum. The radiation dose was determined by the duration of exposure to the caesium source. Since radiation damage seems not to be repaired at low temperatures[16,17], we assume that in these conditions the total radiation dose administered accurately reflects the total damage sustained by the cells.

The hamster line used was Wg3-H, a DON line derivative lacking hypoxanthine guanine phosphoribosyl transferase (HGRT) activity[18]. This cell line generates wild type revertants at a very low frequency, but it was, in any case, propagated in medium containing 6 μg ml^{-1} of thioguanine, which is lethal to wild type cells. Wg3-H cells (3×10^6) were fused with 10×10^6–12×10^6 irradiated lymphocytes by the technique described by Harris and Watkins[1]. Haemagglutinating units (5,000) of inactivated Sendai virus were added to the mixed cell suspensions to make a total volume of 0.5 ml. After fusion, the cells were distributed into 20 small Falcon flasks in MEM. The medium was replaced the following day by HAT medium, which is MEM containing 6×10^{-5} M hypoxanthine, 10^{-5} M thymidine, 4.5×10^{-7} M aminopterin and 4×10^{-4} M glycine. During the medium change, unfused lymphocytes are washed away; unfused hamster cells or fused hamster cells that had not received a lymphocyte component remain deficient in HGRT and are killed in HAT medium; only man–hamster hybrid cells proliferate. No colonies developed in control flasks seeded with hamster cells that had been treated with Sendai virus in the absence of added human lymphocytes. Only one colony was isolated from each experimental flask to ensure that all clones studied were of independent origin.

Table 1 The frequency of cotransference of HGRT with other X-linked markers

Dose krad	Observed frequency of co-transference of HGRT with the unselected markers:			No. of clones examined
	PGK	α-gal	G6PD	
0	0.95	0.95	0.95	46
1	0.80	0.86	0.90	51
2	0.55	0.68	0.75	78
4	0.22	0.34	0.42	77

Species specificity of markers

The four X-linked markers studied were the enzymes HGRT, phosphoglycerate kinase (PGK), glucose-6-phosphate dehydrogenase (G6PD) and α-galactosidase (α-gal). The human forms of all these enzymes can be separated from the hamster forms by electrophoretic techniques. The human forms of HGRT, PGK and G6PD were identified by Cellogel electrophoresis essentially as described by Meera-Khan[19]. The human and hamster forms of α-gal, as detected by hydrolysis of the fluorigenic substrate 4-methyl-umbelliferyl-α-D-galactopyranoside[20], can also be separated by Cellogel electrophoresis in the same buffer as that used for HGRT. Separation of the two forms of the enzyme is simplified if the preparations are treated with neuraminidase[21]. Before electrophoresis, the extracts were therefore incubated at 37 °C for 45 min with an equal volume of neuraminidase from *V. cholerae* (BDH) (500 units ml^{-1}).

Determination of gene order

All clones isolated were assumed to contain the human gene for HGRT. In early experiments, each clone was actually tested for the presence of the human enzyme, but since no revertants containing the hamster enzyme were found, the presence of other human X-linked enzymes was accepted as evidence of the hybrid nature of the clone. The species of origin of the HGRT was checked in all clones that showed no other human enzymes. Table 1 lists the frequencies with which the unselected human markers PGK, G6PD and α-gal were cotransferred with HGRT in the hybrid clones. It is clear that the frequency of cotransference of these markers is reduced as the dose of irradiation adminstered to the human cells is increased. If the human cells are not irradiated, segregation of these markers from HGRT occurs very rarely, and a cytologically identifiable human X chromosome can usually be seen in the hybrid cells. It is very unlikely that direct inactivation of the structural genes by gamma rays, or of other genes governing expression of the markers, contributes significantly to the observed frequencies of segregation; direct inactivation of genes would be expected to occur at a very much lower frequency[22]. The most probable explanation for the segregation of the unselected human markers from HGRT is that the genes coding for these markers are separated from that coding for HGRT by radiation-induced chromosome breaks and translocations. Once separated from HGRT, which is maintained in the hybrid cell populations by selective pressure, chromosome fragments bearing unselected markers would be rapidly lost. If this explanation is, in principle, correct, then the fact that PGK segregates from HGRT more frequently than either α-gal or G6PD indicates that PGK is further removed from HGRT than are the other two markers.

The problem is susceptible to quantitative treatment. We define a segregational event as any event that disrupts the

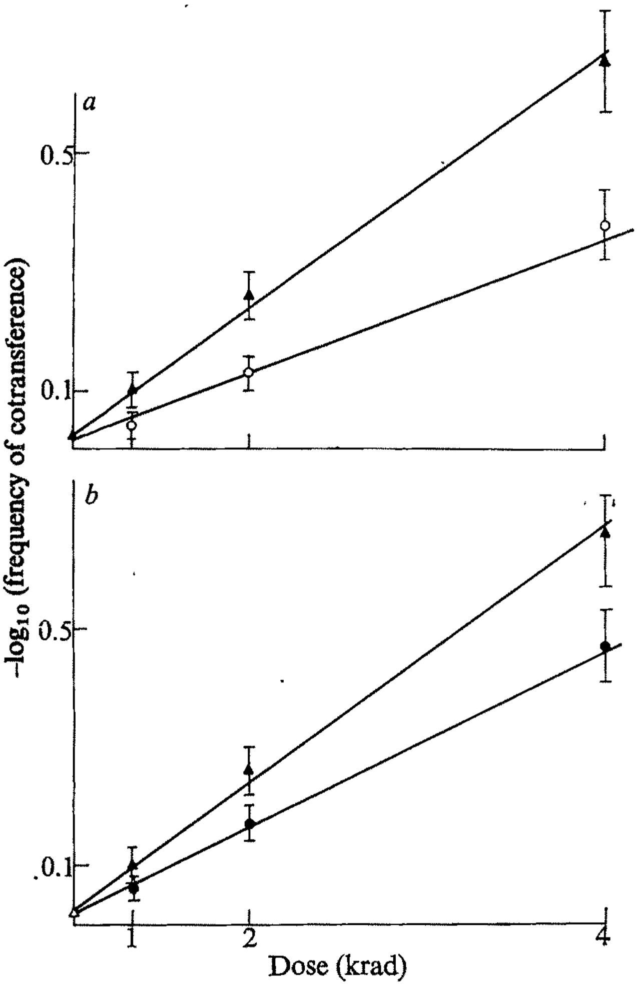

Fig. 1 The logarithms of the frequencies of cotransference of PGK with HGRT, G6PD with HGRT and α-gal with HGRT are plotted against the dose of radiation. The dose axis has been expanded as (Dose$^{1.6}$), an empirically determined function which converts the plots to linearity. The slopes of the lines are proportional to the targets for the segregation of each pair of loci (see text). The target PGK/HGRT is compared with G6PD/HGRT in (*a*), and with α-gal/HGRT in (*b*).

The target sizes calculated relative to PGK/HGRT are: PGK/HGRT = 10 units (arbitrary standard); G6PD/HGRT = 5.4 ± 0.9; α-gal/HGRT = 6.9 ± 1. (Margins of ±1 s.e. are used throughout.) ▲: PGK/HGRT; ○: G6PD/HGRT; ●: α-gal/HGRT.

linkage of two genetic loci. The number, S, of such events induced in the whole genome by irradiation is obviously determined by some function of the dose of radiation delivered. We may therefore write:

$$S = f(D)$$

Events that segregate one locus from another may be considered as occurring within a specific target delimited by the two loci, this target being equivalent to some fraction t of the whole genome. If we assume that, to a first approximation, segregational events induced in interphase chromatin are randomly distributed throughout the whole genome, then the mean number of events $\bar{S}_t$ occurring in target t is given by:

$$\bar{S}_t = t.f(D)$$

The actual numbers of such events occurring in this target will be scattered around the mean value $\bar{S}_t$ with the frequency distribution described by Poisson's theorem. We can therefore use this theorem to predict the probability (P_0) that the number of such events will be zero, that is, the probability that the linkage will not be disrupted:

$$P_0 = \exp[-t.f(D)]$$

If the viability of the hybrid cell is not significantly affected by the segregation of any particular unselected marker, then P_0 can be equated with the frequency F of occurrence of hybrid clones in which the two loci are cotransferred.
Then

$$F = \exp[-t.f(D)]$$

and $$-\log F = t.f(D)$$

This relationship predicts that if the logarithm of the frequency of clones in which two loci are cotransferred is plotted against an appropriate function of the radiation dose, a straight line should be defined with a slope proportional to the size of the target for the segregation of the two loci. Fig. 1 shows that this prediction seems to be correct. The appropriate function o dose was determined empirically to be approximately $D^{1.6}$ and within the limits of experimental error, was found to be the same for any pair of loci. A dose exponent between 1 and 2 has commonly been found for the frequency of chromosome rearrangements induced by X or gamma rays[10]. This exponent indicates that such rearrangements involve a combination o single and double hit events. Calculation of target size does not, however, require an accurate estimate of the dose function Provided that this function is the same for all pairs of loci relative target sizes can be calculated from the ratios of the logarithms of the frequencies of the non-segregant clones. In Fig. 1 the data are plotted for the cotransference of PGK with HGRT (target PGK/HGRT), of α-gal with HGRT (targe α-gal/HGRT) and of G6PD with HGRT (target G6PD/HGRT). The target sizes are calculated relative to the target PGK/HGRT which is arbitrarily assigned a value of 10 units.

If, as we suppose, segregational events are largely determined by chromosomal rearrangements, then one would expect that two unselected markers situated on opposite sides of a selected marker would be segregated from that marker in an independent fashion. Thus, if G6PD and PGK were situated on opposit sides of HGRT, then the segregation of either one of thes markers from HGRT should be independent of the segregation of the other. In Table 2, the observed numbers of clone expressing both the markers PGK and G6PD (P^+G^+), neither marker (P^-G^-) or either marker (P^+G^- or P^-G^+) are compared in each case with the numbers expected if PGK an G6PD segregated from HGRT independently. It will be see that the observed numbers are not significantly different from

Table 2 A test of the independence of the segregation of PGK and o G6PD from HGRT

Class of segregant	Number of hybrid clones in each class at each dose 1 krad	2 krad	4 krad	Total
P^+G^+ expected	37	31.6	7.1	75.7
observed	38	34	10	82
P^-G^- expected	1.0	8.6	35.1	44.7
observed	2	11	38	51
P^+G^- expected	4.0	10.4	9.9	24.3
observed	3	8	7	18
P^-G^+ expected	9.0	26.4	24.9	60.3
observed	8	24	22	54

The number of clones observed in each of the possible classes segregant for the two markers PGK and G6PD is compared wi that expected* if PGK and G6PD segregated independently fro HGRT. $\chi^2 = 3.7$ (calculated from the totals, with 1 d.f.).

*If the overall frequency, at any one dose, for the segregation PGK is p^- and of G6PD is g^-, the number of clones expected in eac class, when a total of n clones is examined, will be: for P^-G^-, $n.p^-g$ for P^+G^-, $n(1\text{-}p^-)g^-$; for P^+G^+, $n(1\text{-}p^-)(1\text{-}g^-)$; for P^-G^+, $n.p^-(1\text{-}g$

the expected numbers ($\chi^2 = 3.7$). We may therefore conclude that the segregation of G6PD from HGRT is essentially independent of the segregation of PGK from HGRT and thus that G6PD and PGK must be on opposite sides of HGRT. Although the differences between the observed and the expected numbers are not statistically signficant, there does seem to be some indication that the segregation of the two markers from HGRT is not totally independent, for the observed numbers of P^+G^- and P^-G^+ clones are consistently higher than expected, and the observed numbers of P^+G^- and P^-G^+ clones are consistently lower. A possible explanation for this marginal effect is discussed later.

A similar analysis of the segregation of α-gal and G6PD from HGRT shows that these two loci must also be situated on opposite sides of HGRT. It then follows that α-gal and PGK must lie on the same side of HGRT and, since the target α-gal/HGRT (6.9) is smaller than the target PGK/HGRT (10), that α-gal must be situated between HGRT and PGK. If this is so, then the segregation of PGK from HGRT must be dependent in part on the segregation of α-gal, for any segregational event occurring between α-gal and HGRT would also segregate PGK from HGRT. Table 3, which is analogous to Table 2, shows that this is indeed the case: the concordant segregants ($P^+\alpha^+$ and $P^-\alpha^-$) occur much more frequently, and the independent segregants $P^+\alpha^-$ and $P^-\alpha^+$) occur much less frequently than would be expected if PGK and α-gal segregated from HGRT independently. In this case, the differences between the numbers of clones observed in each class and the numbers expected are highly significant ($\chi^2 = 36.4$). Our model is thus substantiated and we can confidently deduce from our data that the order of the four genes is:

PGK α-gal HGRT G6PD

This gene order is in accordance with that derived from the concensus of studies involving the use of spontaneous translocations[9].

Target size and map distance

Although the target size for the segregation of two loci is a measure of the map distance between the loci, we cannot assume that the target size is directly proportional to the map distance. This would be so if the only type of event causing the segregation of loci was a chromosome break (or translocation) that separated all the loci on one side of the break from all the loci on the other. One locus could be segregated, however, from another by a locally acting event, for example by direct inactivation of the gene, and the frequency of such an event would clearly be independent of the distance between the two loci. We have already noted that the expected frequency of gene mutation (of the order 10^{-8}) is too low to have any appreciable influence on our results; but it is apparent from our data that a genetic locus can be segregated by a local event of some other kind. For example, Table 3 shows a few cases in which α-gal has been lost while PGK and HGRT are retained, an event formally equivalent to an interstitial deletion. Events of this kind would contribute to our estimates of the target size for the segregation of two loci an increment independent of the map distance between them. We can calculate the effective target size for one such event by scoring the frequency with which α-gal is retained in clones expressing both PGK and HGRT: the calculations yield a target size of 1.7±0.6 units.

There is no reason to suppose that localised segregational events of this kind are limited to the α-gal locus; but with our present data we can attempt to demonstrate their occurrence at only one other locus, HGRT. If the HGRT locus could be separated from the rest of the chromosome by a localised event and incorporated alone in a hybrid cell, then that cell would be scored as one in which all the other three loci had segregated. Thus, occasionally, a single event would simultaneously segregate loci on opposite sides of the selected marker. This effect might explain why, in Table 2, there is some suggestion that the segregation of PGK from HGRT may not be totally independent of the segregation of G6PD from HGRT. The target for such a localised event at the HGRT locus would thus contribute to the targets for the segregation of any other marker from HGRT. This contribution is easily eliminated from the target for the segregation of α-gal from HGRT by recalculating this target from the data on clones in which a localised segregational event at HGRT cannot have occurred, for example, on clones that express G6PD. This new target, α-gal/HGRT(G^+), would still include localised segregational events at α-gal, but we have already calculated the target for these (1.7±0.6), so that the true map distance between α-gal and HGRT is obtained by substracting 1.7 from the target α-gal/HGRT(G^+). The target α-gal/HGRT(G^+) and the analogous targets PGK/HGRT(G^+) and G6PD/HGRT(P^+ or α^+) are then as follows: α-gal/HGRT(G^+) 5.6±1; PGK/HGRT(G^+) 8.3±1.4; G6PD/HGRT(P^+ or α^+) 4.1±0.9. These figures are less than the targets given in Fig. 1, which include the effects of localised segregational events at HGRT, by 1.3, 1.7, and 1.3 respectively. Although these differences, which estimate the target for such events at HGRT, only approach statistical significance with the data we have so far obtained, it seems none the less that this target is similar in size to the target for localised segregational events at α-gal. It seems reasonable to assume that similar targets exist at the PGK and the G6PD loci, but further work is needed to demonstrate this. On this assumption, a provisional statement of map distances would then be: PGK-HGRT: 6.6±1.5; PGK-α-gal: 3.3±1.2; α-gal-HGRT: 3.9±1.2; HGRT-G6PD: 2.4±1.1.

Systematic approach to mapping

The investigations we have described show that the technique of cell fusion coupled with quantitative irradiation of one of the parent cells provides a systematic approach to the mapping of genes within mammalian chromosomes. This approach rests heavily on the presence in a particular chromosome of a selectible marker, although, much more laboriously, it could be applied without such selection. However, since selective procedures exist for genes on chromosomes other than the X and since several X-autosome translocations are available, the technique, without substantial modification, is immediately applicable to a large part of the human genome. A modest number of hybrid clones will reveal the order of the genes, and the accuracy of determination of target size, and hence map distance, is limited only by the number of clones analysed. Karyological analysis is not necessary (except where orientation with respect to the centromere is required) and is thus not a limiting factor in the resolution of the analysis. Since scoring is based on the frequency of non-segregant clones, all clones are informative with respect to all loci studied.

Table 3 To show that PGK and α-gal do not segregate independently from HGRT

Class of segregant		Number of hybrid clones in each class at each dose 1 krad	2 krad	4 krad	Total
$P^+\alpha^+$	expected	35.4	29.2	5.7	70.3
	observed	40	39	12	91
$P^-\alpha^-$	expected	1.4	11.2	39.7	52.3
	observed	6	21	46	73
$P^+\alpha^-$	expected	5.6	13.8	11.3	30.7
	observed	1	4	5	10
$P^-\alpha^+$	expected	8.6	23.8	20.3	52.7
	observed	4	14	14	32

The number of clones observed in each of the possible classes of segregant for the two markers PGK and α-gal is compared with that expected if PGK and α-gal segregated independently from HGRT. $\chi^2 = 36.4$ (calculated from the totals, with 1 d f.).

We thank Dr F. H. C. Marriott for advice on the statistical analysis of our results and Mrs Jean Kerr-Harnott for technical assistance. S.J.G. held a Medical Research Council Scholarship for Training in Research Methods during part of this work and is now a Domus Senior Scholar of Merton College. The work was supported by the Cancer Research Campaign.

Received April 25; accepted May 13, 1975.

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Cholesterol is excluded from the phospholipid annulus surrounding an active calcium transport protein

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When phospholipids in the first shell of the lipid bilayer surrounding a calcium transport protein are replaced by cholesterol, there is a complete and reversible inactivation of ATPase activity. This phospholipid annulus normally excludes cholesterol and the lateral segregation of the lipids is essential for the protein to function.

LIPIDS and proteins can diffuse rapidly in the plane of some membranes at rates of up to several microns per second[1,2]. This fluidity prompts the question of whether persistent interactions can occur between specific proteins and lipids in the bilayer, which normally contains a very heterogeneous mixture of chemically distinct lipids. The question may be acute for a protein that is inserted into a lipid bilayer containing substantial proportions of lipid which will not support its function. To retain function the tertiary structure of the protein must be able to specify an appropriate lipid environment.

Cholesterol is a common component of membrane lipids, and we have found that when it is forced to interact directly with a number of membrane proteins it has a strong inhibitory effect on their function. The structure of the membrane is designed to exclude direct interaction of cholesterol with the protein. This exclusion is caused by a single shell of phospholipid bilayer bound relatively tightly to the protein, which we term the annulus. We are therefore suggesting that the protein imposes a lateral segregation of lipid classes which ensures that the protein is maintained in a functional conformation.

Evidence for the exclusion of cholesterol from the phospholipid annulus is derived primarily from the ATPase which is responsible for the active transport of calcium into sarcoplasmic reticulum (SR). Two calcium ions are taken up into purified SR vesicles for each ATP molecule hydrolysed[3], and 80% of the membrane protein is the ATPase responsible for calcium uptake[4]. Biochemical and spin label evidence led us to propose a structural model in which about 30 phospholipid molecules interact directly with the hydrophobic surface of the ATPase spanning the bilayer, and form the minimal lipid environment required to maintain full ATPase activity of the protein[5,6]. (See also the model proposed previously for cytochrome oxidase complexes with lipid[12].)

The 30 lipid molecules in the annulus can be replaced almost entirely with defined lipids by the technique of lipid substitution[4–7]. All the essential structural features of the protein are conserved, as the pure ATPase–lipid complexes can be incorporated into vesicles which actively accumulate calcium[4]. We have adapted the lipid substitution technique to prepare ATPase–lipid complexes which contain cholesterol. Comparison of the properties of the various complexes provides evidence for the exclusion of cholesterol from the phospholipid annulus.

Cholesterol does not affect activity of ATPase with a complete phospholipid annulus

When phospholipids are added to complexes of pure ATPase with a complete annulus of about 30 SR phospholipids (termed SR–ATPase), there is a rapid and complete equilibration of the phospholipid pools in the presence of appropriate concentrations of cholate[6]. The activity of the ATPase is then determined by the composition of the phospholipids in the annulus: for example, dimyristoyl lecithin (DML) and dipalmitoyl lecithin (DPL) inhibit the activity of pure SR–ATPase complexes to about the same extent, and the inhibition is a linear function of the lipid composition (Fig. 1). In contrast, if SR–ATPase is titrated in the presence of cholate with an excess of dioleoyl lecithin (DOL), DML or DPL, containing increasing proportions of cholesterol, the activity of the equilibrated mixtures is virtually unaffected by the presence of cholesterol at up to equimolar proportions with the added lecithins (Fig 2). If a cholate titration complex of SR–ATPase mixed with equimolar DOL–cholesterol is centrifuged into a sucrose gradient an ATPase–lipid complex is recovered in which the proportion of DOL in the annulus corresponds to at least 90% of the expected substitution of the added DOL for the SR lipid (Table 1). The presence of cholesterol does not therefore prevent the normal equilibration of phospholipids which is facilitated by cholate.

These results are most simply interpreted by assuming that cholesterol does not significantly affect the interaction of the phospholipids with the ATPase, and that the phospholipids exert the same effect on the activity of the enzyme irrespective of the presence of cholesterol. It is possible that a normal interaction of cholesterol with the enzyme is prevented in some way by the use of cholate as the equilibrating agent, perhaps because of the structural similarity between this detergent and cholesterol. This possibility is ruled out by experiments in which cholesterol was introduced into ATPase–lipid complexes by cosonicating SR–ATPase with increasing amounts of equimolar DOL and cholesterol. The resulting ATPase–lipid complexes were collected by centrifugation into a sucrose gradient to separate them from excess lipid which had not become incorporated by sonication. Table 2 shows that a small proportion of the added DOL–cholesterol is fused with the SR–ATPase complexes, and that there is a small increase in the ATPase activity with increasing proportions of DOL–cholesterol in the complexes, similar to that observed by cosonicating with a large excess of DOL alone. Note that the activation of the SR–ATPase by sonication with a 20-fold excess of DOL is similar to that obtained by equilibration of DOL with SR–ATPase by cholate (30% compared with 38% in Fig. 2). To show that the cosonication procedure caused fusion, SR–ATPase was cosonicated with DML or equimolar DML–cholesterol. In both experiments the ATPase activity was reversibly inhibited, indicating a direct interaction of the DML molecules with the enzyme. The inhibition was less than in the presence of cholate because the fusion caused by sonication is incomplete (see ref. 6 for examples of similar experiments). These experiments confirm that cholesterol has no effect on ATPase activity in conditions in which mixing of the phospholipid pools interacting directly with the ATPase can be clearly demonstrated.

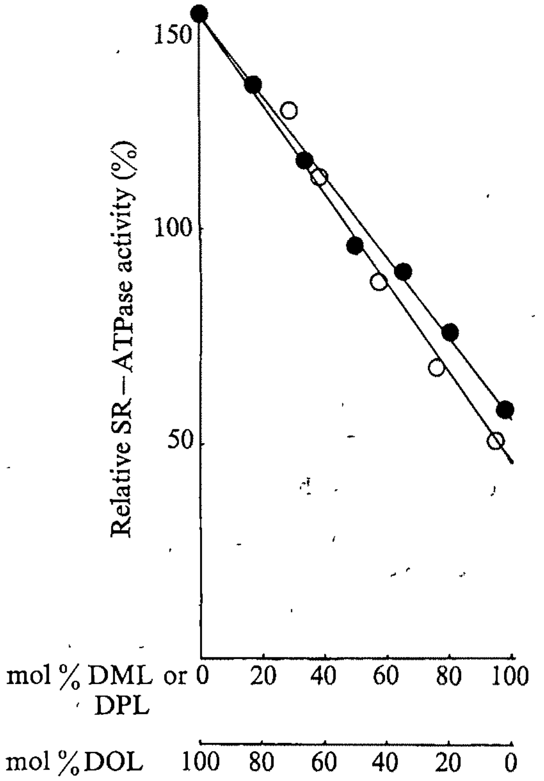

Fig. 1 Titrations of ATPase activity with defined lecithins. SR–ATPase (0.20 mg) was treated with a total of 1 mg phospholipid (DOL–DML or DOL–DPL) and 0.5 mg cholate in 100λ sucrose buffer containing 250 mM sucrose, 1 M KCl, 5 mM MgATP^{2-}, and 50 mM potassium phosphate, *p*H 8.0. After incubation at 40 °C for 10 min, 5λ samples were diluted 200-fold into ATPase assay medium at 37 °C. Added phospholipids comprised 95% of total phospholipid pool and the 100% SR–ATPase activity was 12.0 IU mg^{-1}. Activity of different pure SR–ATPase preparations varied between 12–14 IU mg^{-1} at 37 °C. ●, DML; ○, DPL.

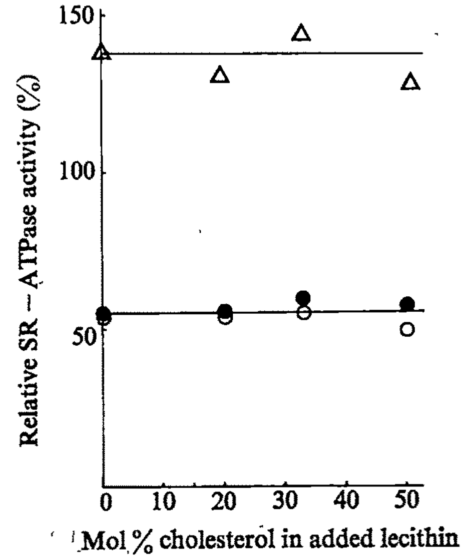

Fig. 2 Titration of ATPase activity with defined lecithin–cholesterol mixtures. SR–ATPase (0.25 mg) was treated with 0.5 mg phospholipid (△, DOL; ●, DML or ○, DPL) containing 0–50 mol per cent cholesterol, and 0.25 mg cholate in 100 µl sucrose buffer. Mixture was incubated at 40 °C and assayed at 37 °C. Added phospholipid comprised 90% of total phospholipid pool.

As cholesterol replaces phospholipids in the annulus ATPase activity is progressively inhibited

To force cholesterol to interact directly with the ATPase, it is necessary to use high concentrations of cholate which strip phospholipids from the annulus[5,6]. If this stripping is performed in the presence of cholesterol, it substitutes for the displaced phospholipids and the enzyme is maintained in a stable but inactive conformation. These complexes can only be reactivated fully by displacing the cholesterol from the annulus by phospholipid.

Fig. 3 shows the relative ATPase activities of 20 separate preparations of ATPase–lipid complexes prepared by treating

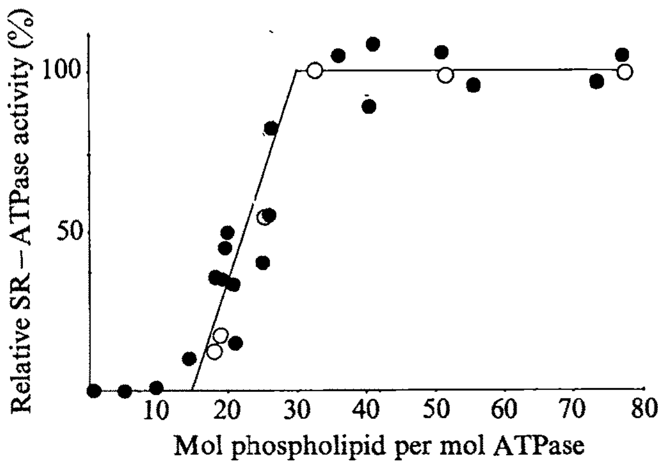

Fig. 3 Effect of replacing phospholipids in annulus by cholesterol. SR vesicles (15 mg) were treated with 0–40 mg cholesterol in cholate at 0.5 mol cholate per mol cholesterol in a total volume of 1.5 ml sucrose buffer. Incubation, isolation and analysis of the complexes was performed as in the legend to Table 1. Cholesterol content of complexes varied between 10–95 mol %. ○, experiments performed in absence of cholesterol.

Table 1 Substitution of phospholipid annulus by DOL in the presence of cholesterol

SR treated with:	ATPase activity (IU mg^{-1}) at 37 °C	Lipid composition of complexes (mol per mol ATPase)			% Substitution of SR phospholipids by DOL	
		Cholesterol	SR lipid	DOL	Expected*	Observed
DOL	13.8	—	3.2	31.5	92	91
DOL+ cholesterol	12.0	24.0	5.4	28.9	92	84

*For complete equilibration of added DOL and SR lipid pools.

SR–ATPase (8.5 mg) was treated with 20 mg DOL, 10 mg cholate±10 mg cholesterol in a total volume of 1.5 ml sucrose buffer. Mixture incubated at 0 °C for 30 min and layered on 2 ml 20% (w/v) sucrose, 0.2 ml 50% sucrose and 0.2 ml 80% sucrose in 1 M KCl and 50 mM potassium phosphate, *p*H 8.0. After centrifugation for 15 h at 5 °C and 200,000g_{av} the supernatant was discarded and the pellet washed with sucrose buffer until the level of cholate had fallen below 0.5 mol cholate per mol ATPase. Lipids were analysed using radiolabelled DOL and cholesterol, and SR lipids by gas liquid chromatography.

SR with cholesterol in the presence of cholate at various concentrations. The complexes were isolated by centrifugation into a sucrose gradient and the residual cholate level reduced to less than 0.5 molecules per ATPase by dialysis. Note that the lipid composition of the isolated complexes depends on the temperature and time of incubation as well as the concentration of cholate, lipid, and ATPase protein (see legend to Fig. 3). Fig. 3 shows that the activity of the complexes is maximal and unaffected by the presence of cholesterol until there are less than about 30 phospholipid molecules per ATPase in the complexes isolated by centrifugation. In all these complexes, the proportion of cholesterol in the lipid varied from 10–95 mol %. Below 30 phospholipid molecules per ATPase there is a sharp drop in ATPase activity, until below about 15 phospholipids per ATPase the complexes have negligible activity. This activity profile is very similar to that observed by increasing the cholate concentration in the absence of cholesterol, also shown in Fig. 3. The important difference is that the complexes inactivated by cholesterol can be reactivated by adding back phospholipid (SR or DOL) in the presence of cholate. Several reactivation experiments are shown in Fig. 4, in which the ATPase–lipid complexes have been titrated with either the precise amount of phospholipid required to complete an annulus of 30 phospholipid molecules, or a much larger excess of phospholipid (about 500–1,000 molecules per ATPase) to achieve maximal reactivation. The partially inactivated complexes can be fully reactivated provided they contain a total of at least 25–30 phospholipid plus cholesterol molecules per ATPase. Complexes with a lower lipid complement prepared by using very high cholate concentrations can only be partially reactivated (Fig. 4). For example, one very inactive complex, containing only 0.7 mol SR phospholipid and 12 mol cholesterol was reactivated 380-fold by a large excess of phospholipid, to 10% of the maximal ATPase activity. Figure 4 also shows that a stoichiometric addition of phospholipid calculated to give a total of 30 phospholipids per ATPase restores 75–80% of the maximal activity of the complex. Furthermore these stoichiometric complexes show a further slow increase in activity over a period of 10–20 h after the initial reactivation.

These experiments show that cholesterol causes a potent inhibition of activity when it replaces phospholipids in the annulus, and that equimolar cholesterol and phospholipid in annulus completely and reversibly inhibits the ATPase. Most of the inhibition caused by cholesterol is abolished by sufficient phospholipid to reform a complete annulus.

Functional significance of phospholipid annulus

The absence of a significant effect of cholesterol on ATPase activity (< ±10%) when there is a complete phospholipid annulus, implies that not more than two of the lipids in the annulus can be cholesterol molecules even when cholesterol and phospholipid are present in equimolar proportions in the membrane. It is clearly essential for the function of the ATPase that it should be shielded from the strong inhibitory action of cholesterol by an appropriate lateral arrangement of the lipids in the bilayer. The similarity of these ATPase results with preliminary data for three mitochondrial enzymes which have been titrated with cholesterol in the presence of high cholate concentrations, suggests that a major class of membrane proteins with diverse biochemical functions will be found to exclude cholesterol from their immediate environment. For example when mitochondria from an ox heart were titrated with an excess of equimolar mitochondrial lipid and cholesterol, the

Table 2 Effect of cosonicating SR–ATPase with DOL–cholesterol mixtures

Molar ratio of DOL–cholesterol to endogenous SR lipid in cosonicated mixture	Lipid composition of the isolated complexes (mol per mol ATPase)			% Relative activity at 37 °C
	Endogenous	DOL	Cholesterol (mol%)	
0:1	29	—	2.5 (7.5)	100
1:1	29	1.9	3.0 (8.8)	98
2:1	24	5.7	7.6 (20)	114
5:1	29	14	17 (28)	110
10:1	28	29	35 (38)	121
20:1	24	69	78 (46)	128
20:1, DOL: SR lipid	26	80	3.0 (2.8)	130

SR–ATPase (7.5 mg) was treated with 0–25 mg equimolar sonicated DOL and cholesterol in a total volume of 1.5 ml 50 mM sucrose buffer. Mixture sonicated under N_2 for 5 min at 20–25 °C and layered on 2 ml 5% (w/v) sucrose and 0.2 ml 50% sucrose in 1 M KCl, 50 mM potassium phosphates, *p*H 8.0. After centrifugation at 5 °C for 1 h at 200,000g_{av} the pellets were collected and analysed as described in Table 1. 100% SR–ATPase activity was 12 IU mg^{-1}. Note that the sonication procedure has little effect on the level of endogenous lipid in the isolated complexes and about 5–15% of the added DOL–cholesterol becomes fused with the SR–ATPase complexes.

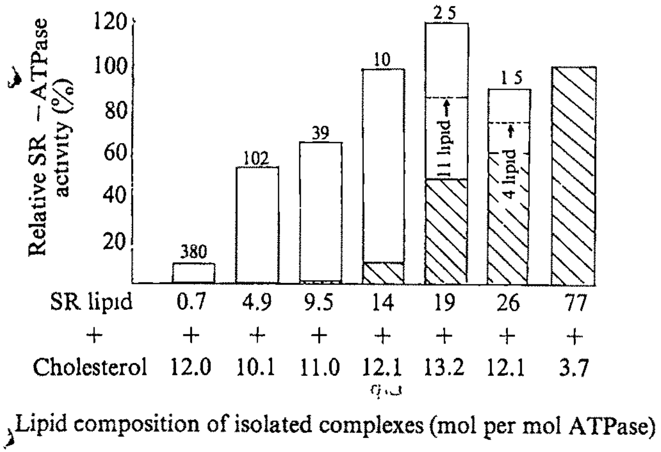

Fig. 4 Reactivation of ATPase by displacing cholesterol bound to protein by SR lipids. Complexes containing cholesterol described in legend to Fig. 3 were suspended at 5–10 mg ml^{-1} protein in sucrose buffer containing 10 mg ml^{-1} cholate and 20–40 mg ml^{-1} SR lipids. After incubation at 0 °C for 2 min the mixture was diluted tenfold into sucrose buffer and incubated for a further 30 min at 0 °C before assay at 37 °C. Shaded area defines activity of complexes before addition of SR lipids; open area, reactivation produced by these added lipids. Factor by which the complexes have been reactivated is shown above each open area. Dashed lines, experiments in which sufficient SR lipid was added back to complete an annulus of exactly 30 SR phospholipid molecules. 100% SR–ATPase activity was 13.5 IU mg^{-1}

activities of the ATPase, succinate dehydrogenase and β-hydroxybutyrate dehydrogenase were all inhibited and the inhibition was fully reversed on removal of cholate. In this respect the lipid interactions of these proteins contrast sharply with the Folch–Lees protein from myelin which was found by London *et al.*[8] to have a strong interaction with cholesterol monolayers.

Cholesterol may be inhibitory because it does not meet the requirement of the protein for specific chemical structures in the annulus: for example, β-hydroxybutyrate dehydrogenase specifically requires lecithin to bind the NAD$^+$ coenzyme[9,13]. On the other hand the very rigid interaction of cholesterol with the hydrophobic surface of the protein would also account for the inhibition of ATPase activity. The reversible inactivation of complexes of the ATPase with saturated lecithins at or below the thermal phase transition demonstrate that a highly rigid interaction is sufficient to completely inhibit the ATPase[6]. The inhibition by both cholesterol and the saturated lecithins can be partially relieved by perturbing agents, such as short-chain alkyl alcohols, which fluidise the lipid environment of the protein[10].

It is probable that entropy factors are responsible for the exclusion of cholesterol from the phospholipid annulus in the ATPase–lipid complexes. It has recently been shown by de Kruyff *et al.*[11] that mixtures of two lecithins which differ by four or more carbons in their fatty acid chains undergo separate phase transitions and that cholesterol is preferentially localised in the more fluid component which has a lower transition temperature. As the annular interaction of phospholipids with ATPase is substantially more rigid and immobilised than the lipid bilayer distant from the protein, a similar mechanism for the exclusion of cholesterol from the annulus is at least plausible.

Finally, we regard the lateral organisation of phospholipids and cholesterol around the protein as a specific example of the way in which the annulus is designed to optimise the accumulation of calcium by SR which will be reported elsewhere.

G.B.W. is the Stothert Research Fellow of the Royal Society; M.D.H. is an ICI postdoctoral fellow. We thank the Science Research Council for a grant to J.C.M.

Received March 20, accepted May 21, 1975.

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letters to nature

Anisotropy of cosmic radiation in the Galaxy

One of the most interesting features of high energy cosmic radiation is its striking isotropy. A large degree of isotropy would be an obvious consequence of an extragalactic origin for cosmic rays, but there are some difficulties in interpreting it in terms of a galactic origin. Although there are theoretical arguments in favour of the latter, the existence of cosmic-ray anisotropy in the Galaxy has not yet been proved experimentally. Here we report a measurement, point M in Fig. 1, which proves the existence of an anisotropy in galactic cosmic radiation. It was detected by examining extensive air showers produced by cosmic-ray primaries of energies ~6×10^{13} eV (which are certainly not affected by solar modulation).

The results of other anisotropy measurements are also shown in Fig. 1. Some of these[1-3] give upper limits only; others[3-6] do indicate some anisotropy. But the Hobart measurement[4] is at low energies for which solar modulation effects are not negligible, and the residual solar waves obtained at Ithaca cast some doubts on the Ithaca points. As for the second Chacaltaya point[3], its statistical weight is rather low and the statistical error given seems to be underestimated. The Pic du Midi experiment[6] also shows some evidence for an anisotropy of about 0.1–0.15% in the energy interval 10^{13}–10^{15} eV. In a later summary[7], however, one of the authors claims to have found no significant sidereal wave at the Pic du Midi.

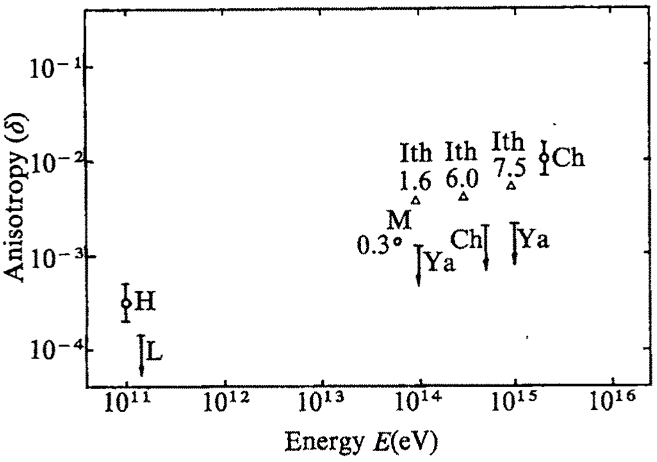


Fig. 1 A plot of experimental results concerning anisotropy, δ, of particle fluxes in the Galaxy as a function of *E*, the particle energy. Figures indicated near the Musala (our work) and Ithaca[5] points are confidence levels (%). H, Hobart[4]; L, London[1]; M, Musala; Ith, Ithaca[5]; Ch, Chacaltaya[3]; Ya, Yakutsk[2].

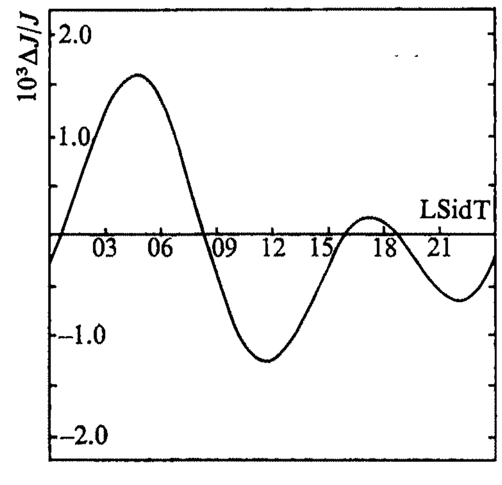
Our apparatus is on the Musala peak (42°N, 24°E) in the Rila Mountains in Bulgaria at an altitude of 2,925 m. The apparatus is of conventional design: four sets of Geiger–Müller counters, each of total sensitive area of 2.5 m², are situated at the corners of a square 8 m × 8 m and connected in four-fold coincidence. The measurement has been running since the autumn of 1968 with temporary interruptions, the largest of which lasted for six months. The counting rate is about 9,000 h^{-1}. Data obtained up to December 31, 1972 were analysed in 3-h bins. The active time of the apparatus was about 11,000 h during which time about 10^8 showers were recorded.

The mathematical method used in the data analysis consisted of a multiple Fourier and simultaneous regression analysis, including barometric pressure and atmospheric temperature. The first three harmonics of solar, sidereal and anti-sidereal daily variations were considered; the flux of the extensive air showers observed was assumed to be of the form

$$J(t) = A+B\Delta p+C\Delta T+\sum_{j=1}^{3}\sum_{k=1}^{3}\{a_{jk}\cos(k\omega_j t)+b_{jk}\sin(k\omega_j t)\}$$

where Δp and ΔT are the deviations of the barometric pressure and atmospheric temperature, respectively, from their mean values. The temperature was measured at the p_0-120 mbar level where p_0 is the actual (variable) value of the barometric pressure taken at the level of observation. The ω_j ($j = 1, 2, 3$) represent the solar, sidereal and anti-sidereal daily frequencies, respectively. Values of the 21 parameters A, B, C, a_{jk} and b_{jk}, together with their errors and correlation coefficients, were estimated simultaneously by the method of maximum probability.

Fig. 2 Sum of the first two harmonics in local sidereal time of the pressure and temperature corrected data obtained in the Musala experiment. ~10^8 showers; $E_0 \approx 6\times10^{13}$ eV.



The relative amplitudes of the solar and anti-sidereal waves remained below noise level (which was roughly 3×10^{-4}). The first two harmonics of the sidereal wave were significant, the confidence level for their superposition exceeding 99.7%. The first harmonic was 7.77×10^{-4} with maximum at 3 h 25 min local sidereal time (LSidT) and the second harmonic had an amplitude of 8.71×10^{-4} with maxima at 5 h 00 min and 17 h 00 min LSidT. The third harmonic was below noise level.

The sum of the first and second harmonics is shown in Fig. 2 and the galactic directions scanned by the apparatus in Fig. 3. There is a maximum between 2400 and 0800 LSidT. In this time interval the detector looks at directions with small galactic declinations; the directions of large fluxes seem to be centred around the galactic anti-centre rather than the inward direction of the galactic arm. There is a minimum between 0800 and 1600 LSidT. In this interval directions close to the galactic North Pole are scanned by the apparatus, and the time interval between 1600 and 2400 LSidT is rather flat.

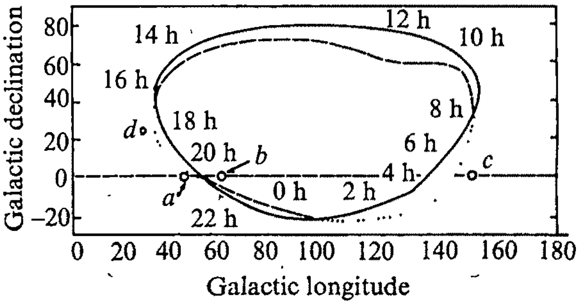


Fig. 3 Galactic directions scanned by the Musala apparatus. Dotted and dashed lines indicate regions of maximal and minimal intensities, respectively. *a*, Direction of spiral in; *b*, direction of galactic rotation; *c*, direction of galactic anticentre; *d*, direction of apex motion.

We note that an isotropic angular distribution of cosmic rays in the Galaxy would result in small sinusoidal solar and sidereal variations because of the orbital motion of the Earth and the apex motion of the Solar System, respectively. Both of these variations are below noise level, at $4.5\times10^{-4}\cos\lambda$ and $3\times10^{-}$ $\cos\lambda$, respectively, where λ is the geographical latitude of the observation. The anisotropy indicates that at least some cosmic ray primaries with energies of about 6×10^{13} eV are of galactic origin.

The absence of a surplus intensity from the direction of the galactic spiral arm indicates that there is no streaming of cosmic rays along the spiral arm at velocities >40 km s^{-1}, which would give rise to a peak in the intensity of the order of 3×10^{-4}.

The intensity maximum observed from the direction of the galactic anti-centre may be understood in terms of an increased density of cosmic rays within the galactic arm. If the Solar System is near the inner edge of the Orion spur then cosmic-ray diffusion produces an anisotropy similar to that observed. On the other hand, we can not exclude the possibility that the anisotropy detected is induced by streaming of cosmic rays in directions out from the Galaxy and perpendicular to the galactic plane (the Sun being to the north from the galactic plane).

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Received February 5; accepted April 24, 1975.

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Structure of irregular galactic magnetic fields

ALTHOUGH there are various methods of investigating experimentally the integral properties of galactic magnetic fields (see, for example, ref. 1), little is known experimentally of the nature of the random component of these fields, at least as far as structures with characteristic lengths $\lesssim 1$ pc are concerned. Here I shall describe a method which, supposing the diffusion approximation of cosmic-ray propagation to be valid, is suitable for determining the structural composition of random galactic fields on the basis of cosmic-ray measurements, down to structures with characteristic lengths ~ 0.001–1 pc.

Magnetic fields, the direction and magnitude of which vary randomly in space, can be characterised by their power spectra, that is, by the function

$$M(k) = \int_{-\infty}^{\infty} C(r)\exp(-i2\pi rk)\mathrm{d}r$$

where $C(r)$ is the coherence function of the random field. I shall show that the form of $M(k)$ can be determined on the basis of the functions $a(E)$, the anisotropy of galactic cosmic rays as a function of energy, and $L(E)$, the total path length of galactic cosmic rays as a function of energy. I shall attempt to apply the procedure on the basis of our present knowledge of the functions $a(E)$ and $L(E)$.

The energy range of the cosmic-ray particles considered is 10^{12} eV $\lesssim E \lesssim 10^{15}$ eV. The lower limit ensures that the energy density of the particle radiation will be low enough to leave the magnetic field traversed unaffected and excludes the possibility that the observed cosmic-ray anisotropy is produced by interplanetary magnetic fields. The upper energy limit excludes regions in which the Larmor radii of cosmic-ray particles may approach the sizes of regions of magnetic irregularities, thus making diffusion processes less effective.

I shall proceed by deriving the energy dependence of λ_p, the diffusion mean free path of cosmic-ray particles in the Galaxy. For simplicity, I assume that the galactic cosmic radiation consists exclusively of protons. There is no intrinsic difficulty in repeating the process for the actual chemical composition of the galactic radiation, except that the chemical composition is very poorly known in the energy interval 10^{12} eV $\lesssim E \lesssim 10^{15}$ eV.

For the simple diffusion process, the fundamental relations between a, L, λ_p and D (the latter being the linear size of the volume to which particles are confined) are

$$D = aL \tag{1}$$

and

$$\lambda_p = a^2 L \tag{2}$$

In the case of the so-called "compound" diffusion process[2], equation (2) has to be replaced by

$$\lambda_m{}^2\,\lambda_p = a^4 L^3 \tag{3}$$

where λ_m is the characteristic length (mean free path) of the 'random walk' of magnetic field lines. In equations (1), (2) and (3) constant factors of the order of unity are neglected.

The functions $a(E)$ and $L(E)$ can, in principle, be determined experimentally. In the preceding paper[3] a recently measured value of $a(E)$ at $E \approx 6\times10^{13}$ eV was reported. Similar measurements, if carried out at various energies, may yield the form of $a(E)$, whereas $L(E)$ can be evaluated on the basis of measurements of the chemical composition of galactic cosmic rays[4–6].

Although serious objections to the simple diffusion model have been raised[2] and the results of Gombosi *et al.* also seem to favour the compound model rather than the simple one[7], I shall investigate the consequences of both models, since they yield the same form for $M(k)$ provided that D does not depend on E.

As to the energy dependence of λ_p, the simple diffusion model obviously yields

$$\lambda_p(E) \sim a^2(E)L(E) \tag{4}$$

In the case of the compound model an assumption has to be made as to the form of $\lambda_m(E)$. I shall suppose that λ_m is independent of E. This seems reasonable on the basis of the assumptions made in the compound diffusion model, in particular that particles are spiralling along field lines while the latter are undergoing random motion in space.

From equation (3) I then have

$$\lambda_p\,(E) \sim a^4(E)L^3(E) \tag{5}$$

I now apply the formula of Jokipii[8] according to which for high energy protons (particle rigidity and particle energy may be interchanged)

$$\lambda_p = E^2/\pi M(k) \tag{6}$$

and $M(k)$ has to be taken at

$$k = 1/r_g(E) \sim B/E \tag{7}$$

where $r_g(E)$ is the gyroradius of a particle with energy E in the magnetic field B in question (resonance scattering).

Equations (4), (6) and (7) yield

$$\text{Simple diffusion: } M(B/E) \sim E^2/a^2(E)\,L(E) \tag{8}$$

whereas equations (5), (6) and (7) give

$$\text{Compound diffusion: } M(B/E) \sim E^2/a^4(E)L^3(E) \tag{9}$$

Equations (8) or (9) yield the form of $M(k)$. If $a(E)$ and $L(E)$ are power functions of E, that is

$$a(E) \sim E^{\eta} \text{ and } L(E) \sim E^{-\beta} \tag{10}$$

then $M(k)$ too will be a power function, since according to equations (8) and (10)

$$\text{Simple diffusion: } M(k) \sim k^{-(2+\beta-2\eta)} \tag{11}$$

and according to equations (9) and (10)

$$\text{Compound diffusion: } M(k) \sim k^{-(2+3\beta-4\eta)} \tag{12}$$

Assuming the average field strength in the Galaxy to be ~ 10^{-6} gauss, protons of energies 10^{12}–10^{15} eV will have gyroradii ~0.001–1 pc. Equations (8), (9), (11) and (12) may thus be applied in the region where

$$0.001 \text{ pc} < k^{-1} < 1 \text{ pc}$$

It is usual to assume $M(k)$ to be of the form $M(k) \sim k^{-\alpha}$ so equations (11) and (12) give the following expression for α in terms of η and β:

$$\text{Simple diffusion: } \alpha = 2+\beta-2\eta \tag{13}$$

$$\text{Compound diffusion: } \alpha = 2+3\beta-4\eta \tag{14}$$

Present experimental results do not enable definite conclusions to be drawn about the values of η and β, that is, the energy dependence of anisotropy and total path length of galactic cosmic rays, respectively. The following crude estimates can, however, be obtained.

On the basis of Fig. 1 of Gombosi *et al.*[3] one may tentatively assume η values like

$$0.3 \lesssim \eta \lesssim 0.5 \tag{15}$$

This estimation is supported mainly by the result of the measurement reported there. Smaller values of η are improbable, for otherwise the London measurement[9] carried out at rigidities around 10^{11} V would have revealed a definite anisotropy instead of an upper limit only, and larger values of η are improbable on similar arguments concerning rigidities of ~10^{15} V (compare Fig. 1 of Gombosi *et al.*[3]).

On the basis of Fig. 3 of ref. 6 one may tentatively assume

$$0.1 \lesssim \beta \lesssim 0.3 \tag{16}$$

in the energy range $1 \lesssim E \lesssim 50$ GeV. Nothing is yet known of the value β in the energy range $10^{12}\text{ eV} \lesssim E \lesssim 10^{15}\text{ eV}$. Assuming, somewhat arbitrarily, that equation (16) remains valid also in this energy range, and putting

$$\eta = 0.4 \pm 0.1 \text{ and } \beta = 0.2 \pm 0.1 \tag{17}$$

one obtains on the basis of equation (13)

$$M(k) \sim k^{-(1.4 \pm 0.2)}$$

and of equation (14)

$$M(k) \sim k^{-(1.0 \pm 0.5)}$$

From equations (1) and (10), one has

$$D(E) \sim E^{\eta-\beta}$$

and, with respect to equation (17)

$$D(E) \sim E^{0.2 \pm 0.15}$$

that is, D, the linear size of the confinement volume depends but weakly on E. Complete independence would prevail if $\eta = \beta$. Present estimates (equation (17)) of η and β do not exclude this possibility; the uncertainties are, however, very large.

I shall, however, assume that D does not depend on E. Equations (13) and (14) show that in this case both equations lead to

$$\alpha = 2-\eta = 2-\beta = 1.7 \pm 0.1$$

that is, both the simple and compound diffusion models yield the same form of $M(k)$. Furthermore, it is interesting to note the agreement of this value with that of $\alpha = 5/3$ imposed by Skilling *et al.*[10] on theoretical grounds.

The applicability of the method described above for the determination of $M(k)$ depends essentially on the validity of the diffusion approximation in describing the propagation of cosmic rays of 10^{12}–10^{15} eV in the Galaxy. Various types of diffusion models may lead to various forms of equations (1) and (2). Two such possibilities ("simple" and "compound" diffusion) have been dealt with in this paper. The validity of equation (6) depends on the role of resonance scattering which, however, may be less important than previously thought[11]. But once the correct forms of equations (1), (2) and (6) are established, the method of determining $M(k)$ can be applied. Calculations carried out on the basis of the very crude experimental data available at present yield sensible results for the form of $M(k)$. Furthermore, there is some indication that the calculated form of $M(k)$ is not too sensitive to details of the diffusion process, that is, the functions $a(E)$ and $L(E)$ may contain (almost) all the information necessary to determine $M(k)$.

I thank Professor R. E. Lingenfelter and Dr R. Ramaty for discussions and the referee for comments.

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Received February 5; accepted May 8, 1975.

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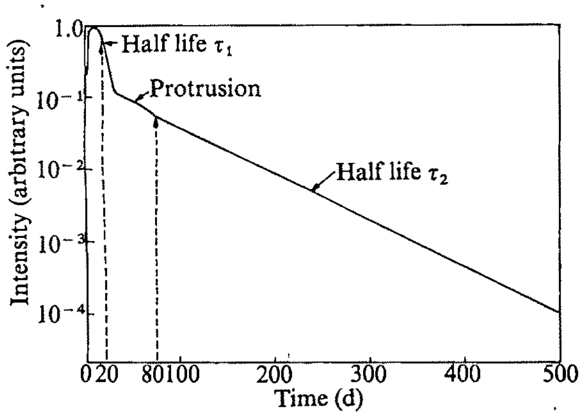
Hypothesis for the Type I supernova light curve

IN a Type I supernova event[1-3] the observed luminosity rises very rapidly to a maximum absolute visual magnitude of about -19.0 ± 0.3. After about 20 d the light curve (Fig. 1) can be characterised by two exponential decays with half lives τ_1 and τ_2 where τ_1 is of the order of several days and τ_2 varies approximately from 40 to 60 d. The integrated observed luminosity is similar for all Type I supernova and is about 3.6×10^{49} erg. A feeble but persistent departure from a simple double exponential decay is also apparent in Fig. 1 and disappears on a time scale τ_3 of ~80 d. We suggest that a model involving ^{56}Ni white dwarf can explain these changes.

Because of its exponential nature it has been suggested that the light curve is associated with the decay in the remnant of explosively produced radioactive species[4,5] such as ^{254}Cf and ^{56}Ni. These hypotheses have fallen into disfavour because the exponential decay times vary substantially from event to event and also because the interstellar medium would become overabundant in heavy elements produced by this process. But our attention has been refocused on such a possibility by the observations which Van Hise[6] made of the supernova in IC4182. He measured $\tau_1=4.8$ d and $\tau_2=56$ d for this event and pointed out that these numbers bear an interesting relationship to the decay sequence of ^{56}Ni. Terrestrially, ^{56}Ni beta decays to ^{56}Co entirely by electron capture with a half life of $\tau_{Ni}=6.1$ d. In turn the ^{56}Co beta decays to ^{56}Fe with a half life $\tau_{Co}=77$ d, 80% by electron capture and 20% by positron emission. The ^{56}Ni decay releases an average total of 1.72 MeV in the form of gamma rays compared to 3.45 MeV for the ^{56}Co decay. Van Hise observed that for the event in IC4182 $\tau_1/\tau_{Ni} \approx \tau_2/\tau_{Co} \approx 0.75$. In other words the light curve looks very much like the ^{56}Ni–^{56}Fe decay sequence but at an enhanced rate. This observation is even more suggestive in the light of explosive nucleosynthesis calculations[7,8] which indicate that the most abundant radioactive element produced by Type I supernovae may be ^{56}Ni with $\sim$ 0.1 $M_\odot$ per event being a reasonable mass estimate.

We propose that each Type I supernova event leads to the formation of a fully convective white dwarf star containing $\sim 0.2 M_\odot$ of ^{56}Ni with a ~ 1 $M_\odot$ remnant expanding away from the star with a velocity[9] $\sim$20,000 km s^{-1} which contains a lesser amount of ^{56}Ni. This hypothesis leads in a simple way to an understanding of the data. The essential idea is that the light curve is powered by the ^{56}Ni decay sequence at an enhanced rate because of the effect of the great matter density in a white dwarf. The electron capture process is proportional to the electron density ρ_e at the nucleus. At ordinary densities the decay of a ^{56}Co or ^{56}Ni nucleus is dominated by the capture of an inner K-shell electron and is relatively insensitive to the state of ionisation or chemical bonding. Using the Hartree–Fock wave functions tabulated by Herman and Skillman[10] we obtain $\rho_e=8.6\times10^{28}$ electrons cm^{-3} for ^{56}Co and $\rho_e=9.7\times10^{28}$ electrons cm^{-3} for ^{56}Ni in ordinary matter. In a white dwarf ρ_e will be increased above the terrestrial values if the average bulk density $\overline{\rho_{av}}$ is comparable to 10^{29} electrons cm^{-3}. The picture here is one of bare nuclei moving through a Fermi sea of degenerate electrons experiencing the average bulk electron density $\overline{\rho_{av}}$. This picture is not perfect since the positive nuclei will tend to attract electrons, the electron density at the nuclei will be increased by some factor above $\overline{\rho_{av}}$. At high electron densities, ρ_e at a cobalt nucleus may be estimated using non-relativistic self-consistent field linear response theory[11] yielding $\rho_e=\overline{\rho_{av}}(1+0.60\times10^{10}\times\overline{\rho_{av}}^{-1/3})$ electrons cm^{-3}. This formula fails where it yields results less than the terrestrial density because K-shell binding occurs. The true value of ρ_e is estimated by smoothly joining these results (see Fig. 2). This gives $\rho_e=1.9\times10^{29}$ electrons cm^{-3} for a matter density $\rho_m\approx2.4\times10^5$ g cm^{-3} and corresponds to a half life of 40 d. This important result may be somewhat modified after more detailed calculations.

Fig. 1 A schematic Type I supernova light curve.



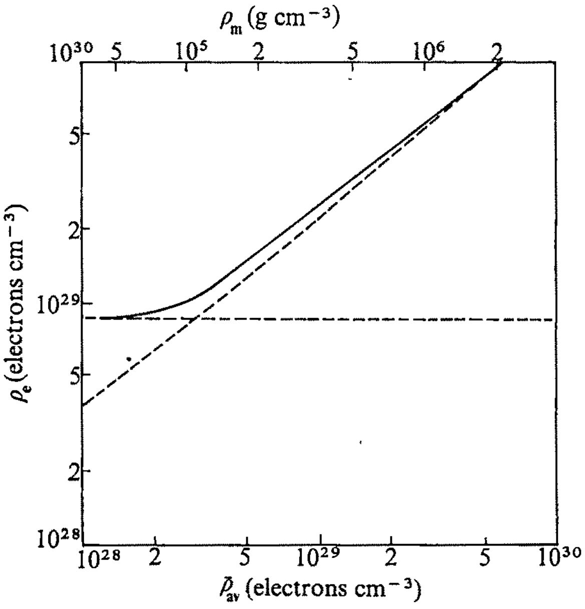


Fig. 2 A plot of the average bulk electron density in a white dwarf $\overline{\rho_{av}}$ as function of the average electron density at a Co nucleus. The linear self-consistent field (LSCF) approximation is used to calculate ρ_e at large $\overline{\rho_{av}}$ (diagonal dashed line). A simple cubic lattice of Co nuclei is assumed. The solid curve is an extrapolation used to make estimates in this paper; the horizontal line marks terrestrial density.

When $\overline{\rho_{av}}$ drops below $\sim10^{28}$ electrons cm^{-3} it is expected that K shell electrons will become bound to the nuclei and dominate the decay. So τ_1 and τ_2 are not expected to become longer than τ_{Ni} and τ_{Co}. Moreover, for an electron density giving $\tau_2=56$ d, the ratios τ_1/τ_{Ni} and τ_2/τ_{Co} should be the same to within 3% when one considers the facts that 80% of ordinary ^{56}Co is being converted by electron capture but that it has a 12% lower electron density than ^{56}Ni.

Since the matter density is expected to vary significantly over the volume of the star one might expect the observed decay rate to be an integral sum of a series of exponentials which itself may or may not seem exponential. But if the star is fully convective on the time scale of $\lesssim$ 1 d then the decay would indeed seem exponential at a rate corresponding to the average electron density at the cobalt nucleus.

More heat per unit mass is generated in the centre of the star than elsewhere, because in the centre the mass density, and thus the decay rate, is greatest. Excess heat will be transported out either by conduction or convection. The convective velocity in the core of a degenerate star has been estimated to be about 10^3 $(L/L_\odot)^{1/3}$ cm s^{-1} (refs 12 and 13). Since the luminosity L varies from about 10^{10} to 10^6 $L_\odot$, a convective mixing time for the present case is about 30 min initially, and several hours after 500 d, thermal conduction times are surely longer, so that violent convection should occur.

No Type I events have been observed with a $\tau_2 \lesssim 40$ d, suggesting that there is an upper mass limit on the ^{56}Ni dwarf formed by the supernova. A τ_2 of 40 d corresponds to an average $\rho_m\approx2.4\times10^5$ g cm^{-3}. For a cold white dwarf[14] we find $M\lesssim0.4\,M_\odot$. This result should be insensitive to chemical composition. But effects not included here (such as rotation and high temperatures) may modify this result considerably. The τ_2 values do not set a lower limit on M, but the integrated observed luminosity does. If the energy released in the radioactive decay is converted to visible light with 100% efficiency then the average dwarf must contain $\sim$0.2 $M_\odot$ of ^{56}Ni.

Because of the rapid convectivity the energy escapes from the star fast enough to allow the light curve to follow the exponentials. Energy emitted from the surface would be a combination of black body photons and energetic particles. If the light curve is to follow accurately an exponential a net mass loss $\lesssim 10\%$ over 500 d is required. Since the peak luminosity of a Tpye I supernova is about 1.2×10^{43} erg s^{-1} the characteristic photon energy is in the soft X-ray region. The remnant should then remain opaque because of ionisation, Compton scattering, and inelastic collisions. Thus we believe that the energy is rapidly deposited in the remnant in the form of electron kinetic energy and converted to visible light by gas collision processes. The diffusion times for visible photons out of the remnant are short enough not to distort the exponential decay.

One convenient explanation of the feeble protrusion in the light curve which disappears on a time scale ~ 80 d is provided by a small amount of ^{56}Ni and ^{56}Co decaying in the remnant. The originally compact remnant is initially opaque to the emitted gamma rays and this energy will also be converted to electron kinetic energy by the Compton process. But as the remnant continues to expand it will eventually become transparent to the gamma rays from the decay. The total absorption coefficient of a 0.5 MeV gamma ray in typical matter is ~ 3 g cm^{-2} and the column density of the remnant falls to ~ 1 g cm^{-2} after 80 d.

We thank M. Ruderman and J. P. Ostriker for discussions, and T. R. Brown, C. Herring, A. Hewitt, P. M. Platzman, R. E. Slusher, and J. A. Tyson for comments.

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Received March 24; accepted April 25, 1975.

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Shock-induced remanent magnetisation in late Precambrian rocks from Lake Superior

A POSSIBLE shock-induced remanent magnetisation, the direction of which yields a typical 'Grenville' pole position has been discovered from late Precambrian Keweenawan igneous rocks (about 1,100 Myr old) on the Slate Islands, situated in northern Lake Superior about 300 miles north-west of the Grenville Front.

The Slate Islands form a cluster of small islets primarily composed of strongly foliated Archaean extrusive and pyroclastic rocks. Along the western shore of Patterson Island, the largest of the group, there are about 450 feet of Keweenawan mafic volcanics, comprising about 20 flows (my unpublished data) that are lithologically similar to other Keweenawan extrusives which border and underlie much of Lake Superior[1,2]. On the Slate Islands the volcanics dip south-west about 30–40°, although toward the base of the sequence dips as great as 50–70° occur. Diabase dikes, trending east to north-east, occur in profusion throughout the islands and are presumably feeders for Keweenawan flows.

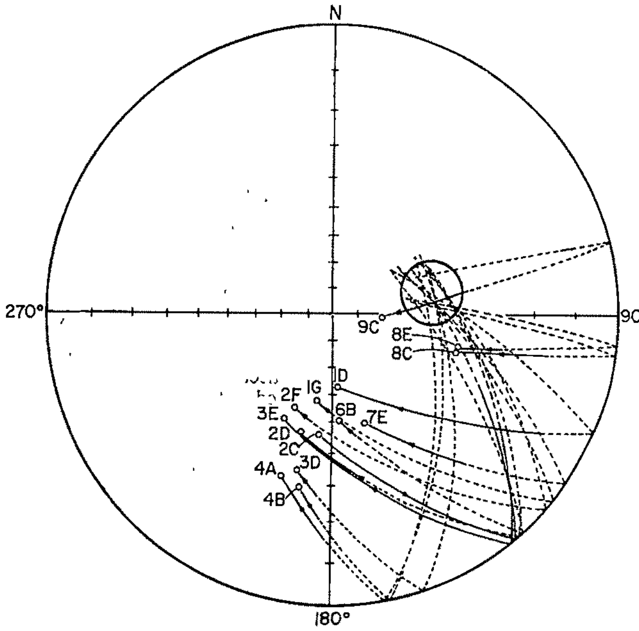


Fig. 1 Wulff stereonet showing remagnetisation circles of samples which yield stable end-points. Some samples have been omitted to avoid congestion. Arrows indicate sense of directional swings on stepwise a.c. cleaning. Data uncorrected for geological dip. ○, Stable end-point (negative inclination); ——, measured curve; - - -, extrapolated curve. Large circle indicates approximate area of convergence.

Two unusual features of Slate Islands geology are a red breccia which cuts all rocks including Keweenawan flows, and conical fractures, known as shatter cones[3] which are genrally thought[3–5] to be shock-induced. The breccia occurs throughout the islands as veins ranging in thickness from less than an inch to several feet, and carries angular to subrounded fragments of host rocks set in a matrix of comminuted rock[6]. Shatter cones are found in both Keweenawan and older rocks from the western half of the Slate Islands but have yet to be recognised in the east where only highly cleaved Archaean rocks occur (R. P. Sage, personal communication). The cones are particularly conspicuous in the Keweenawan flows where they point eastward and have axes inclined about 10° to the east (ref. 6 and R. P. Sage, personal communication).

More than 60 samples were collected at 12 sites from Keweenawan flows and dikes, and oriented using a Sun compass (accuracy ± 2°). Three samples were also taken from a red breccia zone. Samples were cored in the laboratory and specimen remanences measured using a spinner magnetometer (sensitivity 0.02×10^{-6} e.m.u. cm^{-3}). At least one specimen per sample was a. c. demagnetised in steps of 50 or 100 oersted up to maximum peak fields of 1,000 oersted.

Two magnetisation components, A and B, were recognised in all but three specimens on stepwise cleaning, as remanent directions smoothly followed great circle paths on the stereonet (Fig 1). In general, directions progressively moved from smaller to greater declinations and from positive to negative inclinations. About half the specimen attained stable end-points corresponding to remanence A (Table 1). If the circles are extrapolated to lower inclinations, their point of convergence gives the direction of the second magnetisation component B.

The direction of the B remanence was found throug use of variational methods[7] to fit a least squares plane

Table 1 Site palaeomagnetic data for remanence A

Site	N	D_1	I_1	D_2	I_2	k	α_{95}	H
1	5	192.1	−59.2	135.9	−61.6	287	4.5	6–10
2	5	196.8	−48.8	145.1	−63.1	168	5.9	6–9
3	5	200.9	−38.4	173.6	−57.3	46	11.4	6–10
4	2	194.9	−30.0	174.5	−47.6	195	12.1	7–10
5	3	208.1	−44.0	152.6	−70.8	256	7.7	7–10
6	1	173.0	−54.0	120.3	−55.1	—	—	4–6
7	1	162.0	−50.0	124.5	−42.2	—	—	6–9
8	2	104.9	−41.5	104.9	−41.5	4,045	3.9	5–7
9	2	99.9	−59.0	99.9	−59.0	26	50.9	4–6
Mean Site	9			135.0	−58.2	20	11.8	

Pole position for A remanence: 183°W; 56.6°N.

N=Number of samples in which stable end points have been found. A stable end point is defined if the magnetisation direction remains constant within $\pm 1°$ over at least 3 successive a c. demagnetisation field increments spanning at least a range of 200 oersted. H is the average range of peak demagnetising field (in oersted $\times 100$) over which stability is observed. D_1, I_1 and D_2, I_2 are respectively declination and inclination before and after structural correction, in degrees. k and α_{95} are respectively the Fisher precision parameter and the half angle of the 95% confidence cone.

firstly for all remanence vectors defining each sample remagnetisation circle, and then, after averaging to the site level, for all normals to site remagnetisation circles (Fig 2). The normal to the least squares plane in Fig. 2 yields a direction D=85.5°, I=47.5° for remanence B.

All data used to define remagnetisation circles (Table 2) exclude natural remanent magnetisation (NRM) directions or those obtained after a.c. cleaning to 50 or 100 oersted. Below 100 oersted, viscous magnetisation components are often present. Once they are removed, remanence directions seldom depart more than 1°–2° from remagnetisation planes during a.c. cleaning. Larger deviations were only observed in some dike specimens, after the remanence had fallen below about 10% of the initial intensity.

When the data in Fig. 1 are referenced to the palaeohorizontal, remanent vectors, defined by stable end points, yield a mean direction (Table 1) which lies within about 10° of that usually found[8–10] for reversely magnetised Keweenawan igneous rocks (Fig. 3), and which is attributed to a TRM formed during initial magma cooling. Remanence A is thus considered to be primary (Keweenawan). Remanence B must therefore be a secondary component, as suggested also by the observation that in diabase dikes, the B component is dominant in coarse-grained interiors but almost absent in chilled margins where the smaller magnetic grains are likely to have higher coercivities and blocking temperatures and thus be more resistant to remagnetisation. The B remanence, moreover, seems to have postdated differential tilting in the Keweenawan volcanics, because maximum convergence of remagnetisation circles is obtained before any structural corrections are made (Figs 1, 2).

The Slate Islands are unique in that no other Keweenawan extrusive sequence around Lake Superior possesses either shatter cones or a hard prominent secondary remanence. It seems reasonable that the features are related and were acquired during a shock event of presently unknown nature. The remarkable convergence of the remagnetisation circles, considering that maximum orientation errors are possibly 5° after laboratory coring, suggests that the B remanence, unlike the A, was acquired over a time interval short compared with time scales of secular variation. Furthermore, the direction of shatter cones is uniform throughout the Keweenawan sequence and does not reflect differences in structural attitude of flows (R. P. Sage, personal communication). The shatter coning and secondary remanence thus postdate differential tilting, an observation which enhances the idea of a common cause of both.

The red breccia is probably also a product of the shock event, but this conclusion cannot be verified with the scanty palaeomagnetic data available. Of the three breccia samples that were a.c. cleaned, one gave a stable B remanence direction, but the other two, although exhibiting dominant B components, were unstable.

The shock event is suspected to be a meteorite impact because shatter cones are commonly observed in association with rocks whose origin cannot be readily ascribed to normal geological processes[5], and may be formed by shock pressures greater than 20 kbar, values considerably higher

Table 2 Summary of palaeomagnetic data showing remagnetisation circles

Site	N	n	M $D°$	M $I°$	k	α_{95}
1	9	11	30.4°	−29.4°	94	5.3°
2	5	8	48.0	−37.7	400	4.6
3	5	6	62.7	−41.2	31	13.8*
4	3	7	79.5	−37.4	2,302	2.4
5	4	7	46.2	−47.7	92	9.6
6	5	4	44.5	−32.2	204	4.7
7	5	5	28.9	−29.4	469	3.5
8	5	9	7.4	−11.7	272	4.6
9	5	6	− 3.4	−01.9	102	7.6
10	3	7	17.8	−18.8	293	7.1
11	5	5	−11.4	+09.3	100	6.7
12	5	10	27.2	−21.7	24	15.8*

N, Number of independently oriented samples which show clear remagnetisation circles.
n, Average number of data points per sample remagnetisation circle.
M, Mean direction of normals to sample remagnetisation circles (normal to site remagnetisation circle).
k, α_{95}, Fisher statistical parameters for the direction M.

*The large confidence limits arise because at these sites, normals to sample remagnetisation circles are themselves distributed along a plane very close to that given by the dotted line in Fig. 2 (that is the convergence of sample remagnetisation circles to the remanence B direction is even apparent at the within site level).

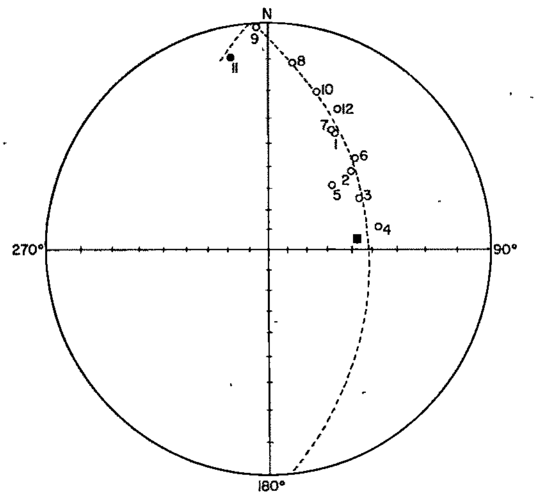
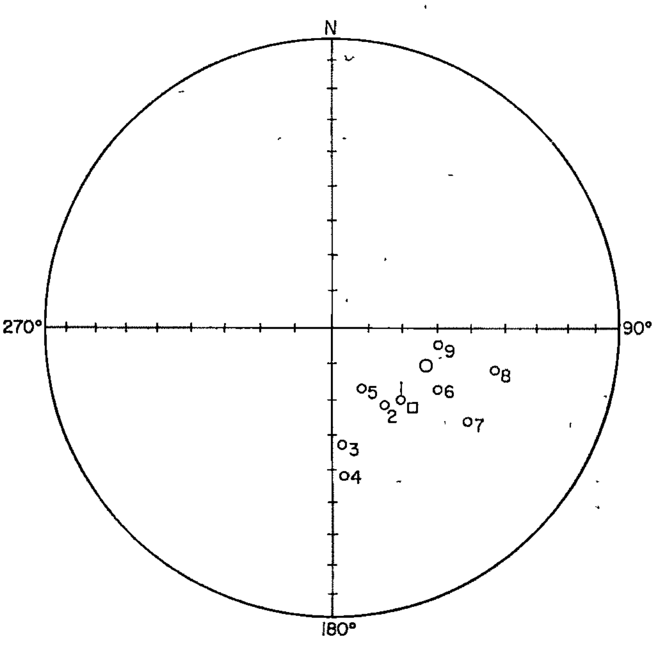


Fig. 2 Wulff stereonet showing the position and normal of the least squares plane fitted to normals of site remagnetisation circles given in Table 2. Solid (open) symbols are plotted on the lower (upper) hemisphere of the stereonet. Data uncorrected for geological dip. ○, ●, Normals to site remagnetisation circles; – – – –, least squares plane through normals; ■, normal to least squares plane (direction of remanence B) with $D=85.5^\circ$, $I=47.5^\circ$.

than the 3–5 kbar maximum estimated for volcanic explosions[3,4]. The attitude of shatter cones along the western side of Patterson Island indicates that the shock centre lay somewhere to the east. The complex geology of the Slate Islands and the presence of a breccia not found on the mainland (R. P. Sage, personal communication), suggest that the shock event occurred locally. Bathymetric charts show a lakefloor depression which almost completely surrounds the islands, inviting speculation that the islands themselves are formed by the central uplift of a meteorite crater, and thus represent the site of impact.

Fig. 3 Equal area projection of site remanence A directions. Data corrected for geological dip. □, Site mean magnetisation direction for Slate Islands volcanics ($D=135^\circ$, $I=58.2^\circ$); ○, Average Keweenawan reversed magnetisation direction.



Several palaeomagnetic studies on breccias and remelted rocks associated with impact sites show[11–14] very stable remanences with low directional dispersion. I am not aware, however, of any report describing a magnetically hard shock remanence in rocks which predate a shock event and which have retained their primary texture. Acquisition of a hard stable remanance in such rocks may either result from mobilisation of domain walls through application of high pressure, or from cooling following a sudden rise in temperature caused by fracturing and adiabatic compression during the passage of the shock wave. The B remanence direction seems unrelated to that of the shatter cone axes and hence to the shock front. It is thus likely to have formed parallel to the ambient palaeomagnetic field.

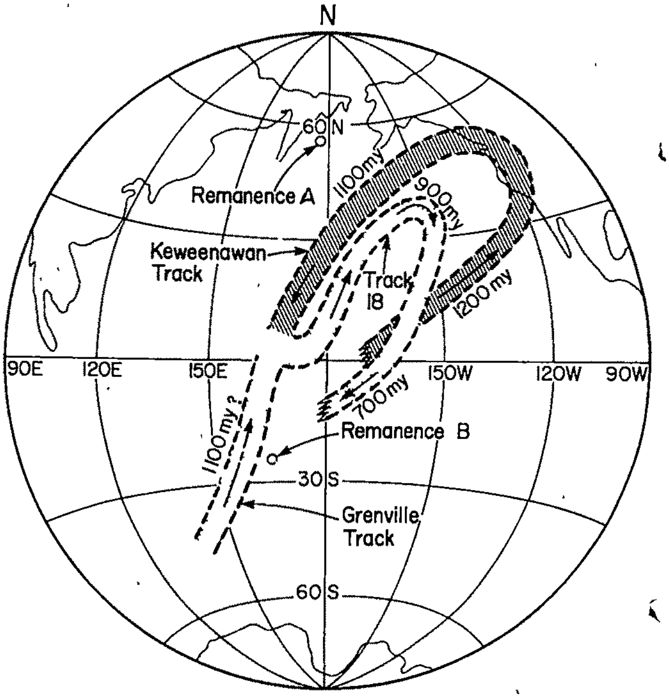


Fig. 4 Map showing the position of the remanence A and B poles relative to the polar wander tracks described[15] for the time interval 1,200–700 Myr ago. The A pole does not lie directly on the Keweenawan polar path, possibly because the very thin flow sequence on the Slate Islands represents a period of time too short for complete averaging out of secular variation.

Palaeomagnetic studies on rocks from the Grenville Province[15–17] reveal stable magnetisations which are believed to be the result of metamorphism and subsequent cooling through magnetic oxide blocking temperatures. Irving *et al.*[15] have summarised all recent data and conclude that the formation of stable Grenville magnetisations occurred about 1,150–1,000 Myr ago, a time interval represented by rocks in other parts of North America (such as the Keweenawan). The Grenville polar wandering path, however, is very different from that of supposedly contemporaneous Keweenawan units (Fig. 4) and it has been proposed[15] that Grenville magnetisations were acquired when the Grenville Province was separated from the rest of North America. If remanence B records a palaeofield direction (albeit at a relatively instantaneous point in time) it would yield a palaeomagnetic pole (24°S, 166°E) which lies about half way along the Grenville polar track (Fig. 4). Remanence B may thus have been acquired during the cooling stage of Grenville metamorphism. This interpretation, if correct, would suggest that the Grenville Province has always remained part of North America, and furthermore, that Keweenawan igneous activity preceded acquisition of stable Grenville remanences. An alternative explanation for the B remanence is that it is much younger than the Grenville Province, perhaps about 700 Myr old, and represents a southerly extension of polar track 18 of Irving *et al.*[15] (Fig. 4).

This research was supported by a grant from the National Research Council of Canada.

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Received February 18; accepted March 5, 1975.

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Alkyl substituted cyclic ethers in 2,300 Myr old Transvaal algal stromatolite

Two cyclic ethers, 2-*n*-propyl-3-methyltetrahydrofuran and 2-*n*-propyltetrahydropyran, have been identified for the first time from the insoluble polymer-like kerogen in a Precambrian rock by ozonolysis, gas chromatography and mass spectrometry. These compounds could prove to be the oldest indigenous biochemical fossils. This same carbonate stromatolite contains abundant and well preserved fossil coccoid and filamentous blue–green algae, some of which show the first known evidence of the diversification of cells[1]. Many of the filamentous algae in this and other Transvaal stromatolites contain specialised cells, akinite and heterocyst-like structures[1,2]. This sample was obtained 750 m stratigraphically above the base of the Transvaal Sequence from an outcrop approximately 315 km north-east of Johannesburg, South Africa[3]. Using isotope dating[4,5] the age of this sample has been estimated to be ~2,300 Myr (D. A. Pretorius, personal communication).

To remove all possible contamination before analysis the surface layer of the sample was removed with a high speed drill, sonicated in triple distilled water, and finally pulverised and extracted consecutively with distilled benzene–methanol, methanol and water. This removes non-indigenous soluble substances which can invade even dense rocks[6–8]. Next, the carbonate rock was dissolved in distilled HCl, and the residue was re-extracted with the same organic solvents as before. This residue was then ozonised, treated with 30% H_2O_2, and extracted with freshly distilled diethyl ether[9]. A complete blank experiment using ignited basalt powder and the evaporation residue of 1,000 ml of the diethyl ether did not contain any cyclic ethers or other organic compounds.

The ether extracts were analysed by combined gas chromatography–mass spectrometry. A 100 foot×0.02 inch internal diameter OS–138 (support coated open tubular, SCOT) column was the most effective for separating the two major peaks. Four other SCOT columns, three 150 foot× 0.02 inch and one 100 foot×0.02 inch, Apiezon–L, SE–30, Dexsil–300 and OV–17, respectively, were evaluated but found to be less effective. Two major components were eluted and partially separated from the ether extract by the OS–138 column. A single focusing Hitachi-RMU-6E mass spectrometer provided rapid scan mass spectra for each component. The mass spectrum of the first major eluate is shown in Fig. 1*a*. In Fig. 1*b* we show a composite mass spectrum consisting of a mixture of 75% 2-*n*-propyl-3-methyltetrahydrofuran and 25% 2-*n*-propyltetrahydropyran standards, because it was established by extrapolation that the first major gas chromatographic peak (corresponding to 2-*n*-propyl-3-methyltetrahydrofuran) overlapped the second major peak by 25%.

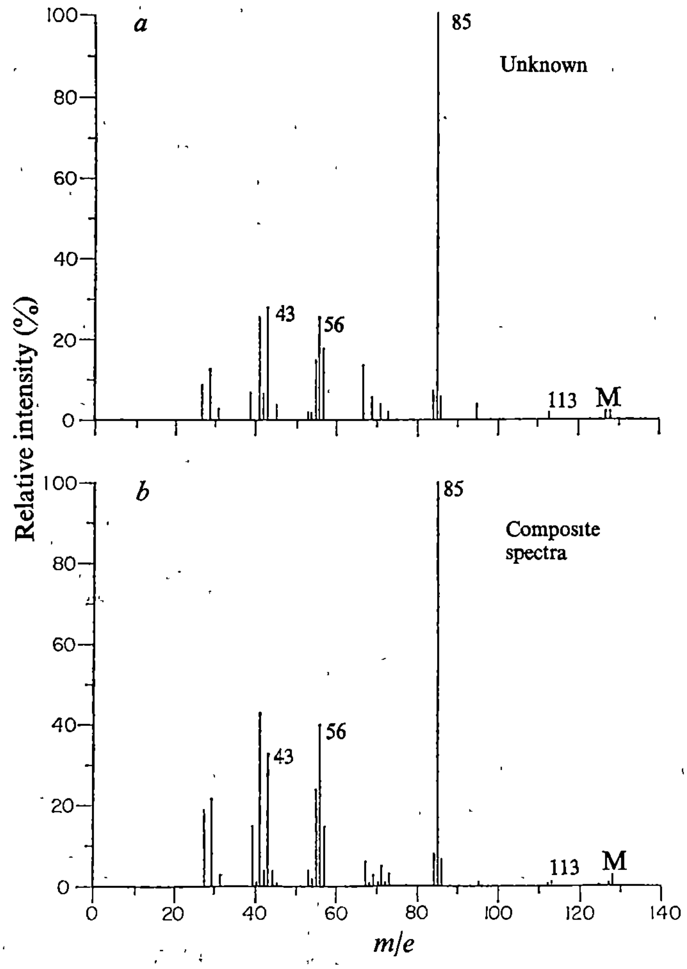

Fig. 1 *a*, Mass spectrometric fragmentation pattern of the first of two prominent gas chromatographic peaks of the ether extract of ozonised Transvaal stromatolite kerogen. This peak is overlapped and contains 25% of the adjacent, second prominent peak. 70 eV. *b*, Composite mass spectrum consisting of 75% 2-*n*-propyl-3-methyltetrahydrofuran and 25% 2-*n*-propyltetrahydropyran. 70 eV. M, $m/e = 128$.

This composite mass spectrum closely resembles the mass spectrum of the first major gas chromatographic peak. The mass spectrum of the second peak also corresponds to a composite spectrum of the same standards containing considerably more 2-*n*-propyltetrahydropyran.

Figures 2*a* and 2*b* show the mass spectra of the 2-*n*-propyl-3-methyltetrahydrofuran (1: 1 mixture *cis* and *trans* isomers) and 2-*n*-propyltetrahydropyran standards, respectively. Both the unknown components and the standards have identical parent ions ($m/e=128$) and base peaks ($m/e=85$). α-Cleavage of the propyl substituent on tetrahydropyran or methyltetrahydrofuran gives rise to the characteristic $m/e=85$ base peak[10]. High resolution mass spectrometry of the unfractionated diethyl ether extract

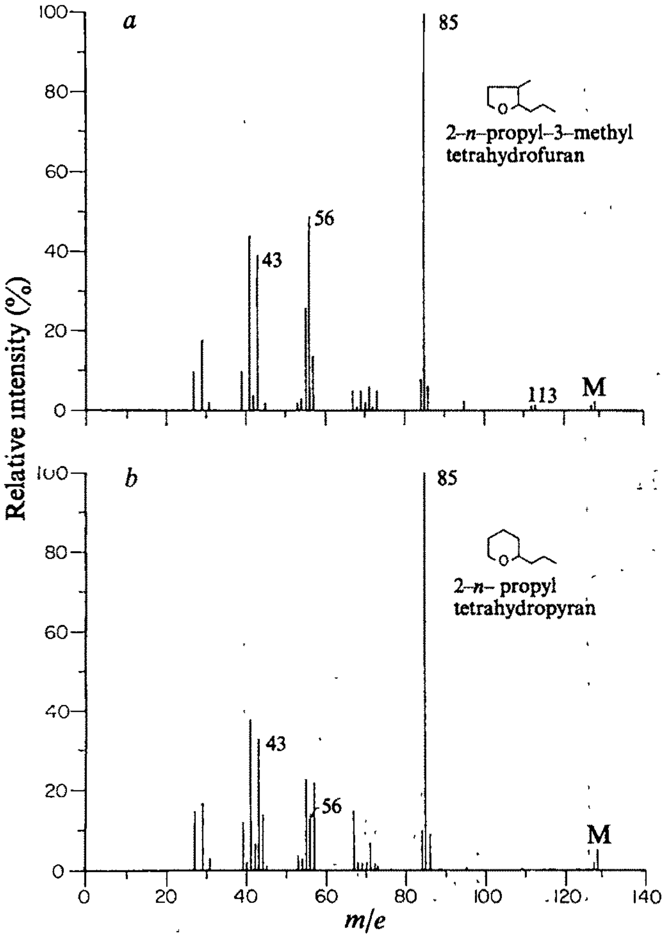

Fig. 2 Mass spectrometric fragmentation patterns of: *a*, 2-*n*-propyl-3-methyltetrahydrofuran; *b*, 2-*n*-propyltetrahydropyran standards. 70 eV.

showed many ions, but only one for the composition $C_8H_{16}O$, which is the parent ion (M) of methyl, propyltetrahydrofuran and propyltetrahydropyran ($m/e=128.11869$).

The M–15 ion (loss of CH_3) was established, $m/e=113.09660$, corresponding to $C_7H_{13}O$. The base peak gave two compositons, $m/e=85.06570$ and 85.02839, correspond-to C_5H_9O and $C_4H_5O_2$, respectively. The latter, unrelated to the methyltetrahydrofuran and tetrahydropyran fragment ions, was much less abundant than C_5H_9O. Thus, 2-*n*-propyl-3-methyltetrahydrofuran and 2-*n*-propyltetrahydropyran were identified by mass spectrometry in the ether extract of the ozonised kerogen from the algal stromatolite.

The identification raises three questions: are the cyclic ethers ozonolysis artefacts; are they part of kerogen since they do not contain the characteristic carboxyl groups[8] of ozonolysis degradation products; and what is their origin? The first possibility is unlikely because these cyclic ethers are not known to be ozonolysis artefacts arising from alkanes during the relatively mild procedures applied[11] (G. A. Hamilton, personal communication). Furthermore, ozonolysis of double bonds will result in *cis*-cycloaddition of O_3 causing bond rupture. The second question is more difficult to answer but the two cyclic ethers, liquid at room temperature, should have been removed by solvent extractions if they were not trapped before and after the dissolution of the rock. Perhaps they were trapped mechanically and/or held by weak bonding inside the kerogen matrix.

Accounting for the origin of these cyclic ethers forces one to propose a hypothesis which should be geologically, biologically and chemically plausible because these compounds occur in a rock built by[12] and containing abundant filamentous blue-green algae. The major component o some of these specialised cells are carbohydrates (on a dr weight basis) yet the cyclic ethers do not have carbo skeletons identifiable with common biochemicals. (Mos coal degradation products do not have biochemical carbo skeletons either.) One of the many possible mechanisms fo their biological origin is suggested: acid converts hexoses such as glucose, to 5-hydroxymethylfurfural[13]. Aci catalysed condensation with pyruvic or oxalacetic acids important biochemicals associated with the metabolism o carbohydrates, could yield 2-(3-keto-3-carboxylprop-1-ene)-5-hydroxymethylfuran[14,15]. During diagenesis, reduction o keto and olefinic groups may occur by hydrogenation accompanied by decarboxylation[16]. The 5-hydroxymethy group on the resultant 2-*n*-propyltetrahydrofuran moiet could form a primary carbonium ion in the presence o alumina gel (and certain clays) with subsequent rearrangement yielding the more stable secondary carbonium io leading to 2-*n*-propyl-4,5-dihydropyran[17]. Further reductio could produce 2-*n*-propyltetrahydropyran.

Catalytic O_2 oxidation (perhaps from algal photosyntheti O_2) or microbial oxidation of the 5-hydroxymethylfurfura could yield 5-carboxylfurfural. Condensation with pyruvi or oxalacetic acid would yield 2-(3-keto-3-carboxylprop-1-ene)-5-carboxylfuran. (Thus, the 2- and 5-positions on th furan ring are blocked, and the 4-position is deactivated b the carboxyl group.) Formaldehyde, an oxidation produc of methanol and breakdown product of certain amino acids in the presence of HCl (one component[18] of known, nearby simultaneous volcanic events[3]) may provide the methy group in the 3-position[19]. Subsequent reduction and decarboxylation[16,20] during diagenesis could yield the 2-*n*-propyl-3-methyltetrahydrofuran.

The suggested pathways for the evolution of both cycli ethers first require a biochemical and subsequently a geological environment. The precursors and, therefore, th products of these suggested reaction mechanisms woul be compatible with the biochemistry of the modern blue green alga, *Raphidiopsis*. This alga is analogous[1] to th oldest known complex life forms found in this sam sample.

We thank Professor Klaus Biemann of MIT for the hig resolution mass spectrometric analyses and for comment Professor Richard S. Glass for contributing to the reactio mechanisms, Dr Keith A. Kvenvolden, Mrs Lois A. Nag and Dr Anthony R. Michaelis for comments. This work wa supported by NASA.

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Received March 3; accepted May 5, 1975.

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Seasonal high and low density bands in reef coral skeletons

THE skeletal aragonite deposited by scleractinian reef corals is characterised by alternating bands of high and low density carbonate that are concordant with the growth surface of the colony. These density variations, which are readily observed by X-radiographic examination of 5–10 mm thick sections of the corallum cut parallel to the axis of growth, are annual in periodicity, such that one high density band plus one adjacent low density band represent the skeletal increment formed over one calendar year[1-4]. Skeletal X radiography is a powerful tool for studying both modern and fossil corals, for in addition to providing a wealth of growth rate data from specimens that grew undisturbed for many years in their natural habitat on the reef, valuable information about the coral itself and about the environment in which the coral lived can be obtained. As Buddemeier[5] points out, density banding in reef corals can not only yield physiological and ecological information about these marine organisms, but also help to unlock the record of long term environmental conditions that is embodied in a coral skeleton. Reef corals, for example, are effective samplers of certain important radionuclides[6].

Although a density band pair clearly corresponds to an annual increment of carbonate deposition, it has not yet been determined when during a year a growth band of particular density actually forms. Thus until a quantitative relationship between density patterns and environmental parameters is established either on an empirical basis or through an improved understanding of coral physiology, the full potential of reef corals in reconstructing climatic history of the recent past cannot be realised[5]. Different attempts to determine when, or during what season, a high or a low density band forms have led to remarkably contradictory conclusions. Some investigators[4,6-8] suggest that the high density band is associated with seasonal low water temperature (such as during Northern Hemisphere winter), whereas others[2,9,10] report that high skeletal density is correlated with periods of high water temperature.

The techniques used so far to resolve this problem involve either: incorporating a fiducial time marker in the skeleton of a living coral (perhaps by *in situ* staining with alizarin red S or similar dyes at a particular time of the year), enabling the animal to continue growing for an extended period, and finally examining the skeleton by X radiography[7]; or correlating the density of the outermost (last formed) growth band with the time of collection of living corals (and thus with environmental parameters such as water temperature and illumination levels) using a large number of specimens removed from both Northern and Southern Hemisphere reef localities on known dates[9,10].

A different approach, described here, is based on the fact that the oxygen isotope ratio of the skeletal carbonate is strongly temperature dependent[11,12], larger $^{18}O/^{16}O$ ratios being associated with lower water temperatures. Isotope ratios are expressed in conventional notation as $\delta^{18}O = 1{,}000\,(R_x/R_s - 1)$, in ‰, where R is the $^{18}O/^{16}O$ ratio; x and s refer to sample and standard (PDB CO_2) respectively. For $\delta^{13}C$, R in this above equation corresponds to the $^{13}C/^{12}C$ ratio. Using the subscripts c and w to indicate skeletal carbonate derived from coral and seawater respectively, $\delta^{18}O_c$ is determined by three factors: temperature (T) of deposition; $\delta^{18}O_w$; and the relative amounts (A) of carbonate oxygen obtained from two different sources, bicarbonate dissolved in seawater, and metabolic carbon dioxide[13]. The effect of $\delta^{18}O_w$ can be eliminated using corals from localities where seasonal changes in $\delta^{18}O_w$ are almost entirely absent. Thus if variation in $\delta^{18}O_c$ due to variation in A can be removed, the remaining variation in $\delta^{18}O_c$ can be attributed to variation in T. In this way it is possible to relate $\delta^{18}O_c$ to density differences between growth bands in a coral skeleton, and thus to relate such density differences to T.

Because $\delta^{13}C_c$ and $\delta^{18}O_c$ are positively (directly) correlated as a result of simple mixing of two components (HCO_3^- enriched in ^{13}C and ^{18}O; metabolic CO_2 enriched in ^{12}C and ^{16}O)[13], it is necessary to measure only three variables simultaneously in order to establish a unique relationship between T and skeletal density (D). These variables are: (I) $\delta^{13}C_c$; (II) $\delta^{18}O_c$; and (III) D. From a set of such data adequate in the statistical sense, the influence of A can be eliminated by partial correlation analysis[14] by calculation of $r_{\text{II III}\,\cdot\,\text{I}}$, that is, the coefficient of correlation between variables II and III independent of variable I.

For an investigation of this sort, it is desirable to examine corals from localities characterised by rather large seasonal changes in water temperature, as $\delta^{18}O_c$ differences between high and low density bands will be correspondingly large, and experimental error involved in eliminating the effect of A will be minimised. This qualification, however, must be balanced by the fact that the thickness (width) of the density bands decreases with decreasing mean annual water temperature[15,16], that localities with large seasonal changes in water temperature also have relatively low values for mean annual water temperature, and that a finite quantity of skeletal carbonate is required to carry out an isotopic analysis.

After X-radiographic examination of 1,488 specimens of modern reef corals representing 31 reef localities widely distributed over the Indo–Pacific and Caribbean regions, a large colony of *Porites lutea* from Heron Island, Australia (23 °S 152 °E), was selected for detailed study. At this offshore site on the southern Great Barrier Reef, winter to summer monthly water temperature ranges between 20.9 and 26.5 °C. An X radiograph of this specimen was used as a guide in locating the position of 45 subsamples removed from a section of corallum with a vanadium–steel cylindrical cutter 3 mm in diameter. After drilling the 45 holes, the section was again X radiographed to confirm the precise location of the subsamples with respect to the density bands. Isotope ratios were determined by high-precision mass spectrometry[17,18] using analytical techniques providing reproducibility of 0.04‰ (standard deviation) for $\delta^{13}C$, and 0.05‰ for $\delta^{18}O$. D is expressed as the percentage of the high density band included in the subsample; as the diameter of the cutting tool slightly exceeds the width of the high density band, values of D were restricted to the range 0–75%.

Correlation coefficients calculated from these data for the three variables defined above are: $r_{\text{I II}} = 0.5860$; $r_{\text{I III}} = 0.1908$; $r_{\text{II III}} = -0.1259$. The partial correlation coefficient[14] $r_{\text{II III}\,\cdot\,\text{I}}$, which expresses the degree of correlation between $\delta^{18}O_c$ and D independent of $\delta^{13}C_c$, is -0.2989. With 42 degrees of freedom, this value of r is statistically significant at the 5% level. As indicated by the negative sign, the correlation between $\delta^{18}O_c$ and D is negative (inverse), that is, greater skeletal density is associated with lower $^{18}O_c$ content and thus with higher water temperature. On this basis, we conclude that the high density band in a reef coral skeleton forms during that time of the year when water temperatures are relatively high. This supports our earlier conclusion from a study of the density of the outermost growth band in 1,488 X-radiographed coral specimens that were collected on known dates[9,10].

Research supported by the US National Science Foundation.

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Possible occurrence of marsupial bones in Cretaceous eutherian mammals

MARSUPIAL bones (epipubes, prepubes) occur in Marsupialia, Monotremata and have also been found in Cretaceous Multituberculata[1]. Gregory and Camp[2] and Romer[3] supposed that prepubic cartilages existed in therapsids. Fourie[4,5] described a tritylodontid skeleton in which these bones were preserved. Marsupial bones have not been found in eutherian mammals unless one accepts Jellison's[6] idea that the baculum is homologous to the epipubic bones. The earliest known eutherian postcranial skeletons come from the Djadokhta Formation (?Santonian) and Barun Goyot Formation (?middle Campanian) in the Gobi Desert, Mongolia[7,8] (and are now in the Zaklad Paleozoologii, Warsaw). The most complete are the skeletons of *Zalambdalestes* and *Barunlestes*[8], both assigned to the family Zalambdalestidae. Study of the structure of their dentition and skulls leaves no doubts that the Zalambdalestidae are members of the Eutheria.

A partial skeleton of *Barunlestes butleri* Kielan-Jaworowska, from the Barun Goyot Formation, Khulsan locality in the Gobi Desert, Mongolia (No. MgM-1/77) includes the right os coxae, the ventral margin of which is not complete. The anterior margin of the acetabular branch of the pubic bone has a structure unusual for eutherian mammals. On the lower part of this margin a triangular, concave surface is arranged at an obtuse angle to the outer surface of the pubic bone. The triangular concavity is elongated dorsoventrally, pointed downwards, with a tubercle on its upper anterior corner and a medial longitudinal groove. The concavity is separated from the outer surface of the pubis by a distinct ridge, more prominent dorsally than ventrally. This triangular fossa is interpreted to be an articular surface for a marsupial bone.

It is not clear from the description and photographs presented by Fourie[4,5] whether a fossa is present on the anterior margin of the pubis in Tritylodontidae. In Cretaceous Multituberculata the articular surface is absent from the anterior margin of the pubis. In Monotremata (Fig. 1*a*) there is a distinct, oval fossa on the lower part of the anterior margin of the pubis, which articulates with the thickened posterior border of the marsupial bone. Anterodorsal to the fossa there is sometimes a small apophysis, which articulates with the posterodorsal apophysis of the marsupial bone. The shape of the fossa, which is very distinctly outlined and deep, is different from that in *Barunlestes* (Fig. 1*b*). Postcranial skeletons of Cretaceous Marsupialia have not been recognised and it is as yet impossible to conclude whether such a fossa occurred in early marsupials. In modern marsupials the articular area for the marsupial bone strongly varies in shape. In most marsupials known to me there is no fossa on the anterior edge of the pubis, and in some families (for example,

an oval condyle. In the Dasyuridae (for example, *Dasyurus*, Fig. 1*c*) at the lower part of the anterior edge of the pubis, however, there is a small, shallow, irregular fossa, comparatively shorter than in *Barunlestes*. These comparisons suggest that marsupial bones may have occurred in the Cretaceous eutherian *Zalambdaletes*.

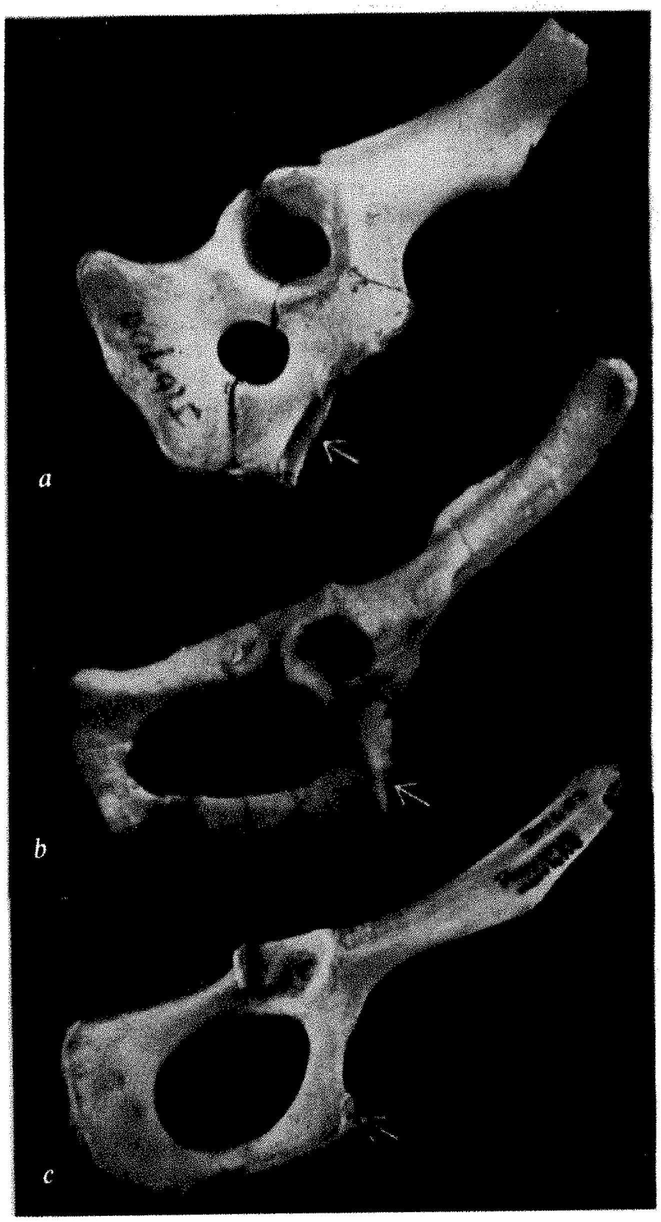

Fig. 1 Right os coxae. Arrows denote the articular surfaces on the pubis. *a*, *Tachyglossus* (×1.4); *b*, *Barunlestes* (×3); *c*, *Dasyurys* (×1).

The presence or absence of the marsupial bones in the extinct orders Triconodonta, Docodonta, Symmetrodonta and Pantotheria cannot as yet be determined because the postcranial skeletons have either not been found or not yet described (Morganucodontidae). It is possible, however, that marsupial bones were present in all these early mammals. Disappearance of marsupial bones in the Tertiary Eutheria may be connected with the gradual evolution of prolonged internal gestation period.

The pelvis in mammals is variously shaped according to the mode of reproduction. It is widely open posteriorly and the ischial arc is U-shaped in Monotremata (Fig. 2*a*), possibly in connection with oviparity. The ischial arc is V-shaped in Marsupialia (Fig. 2*b* and *c*), but the angle at

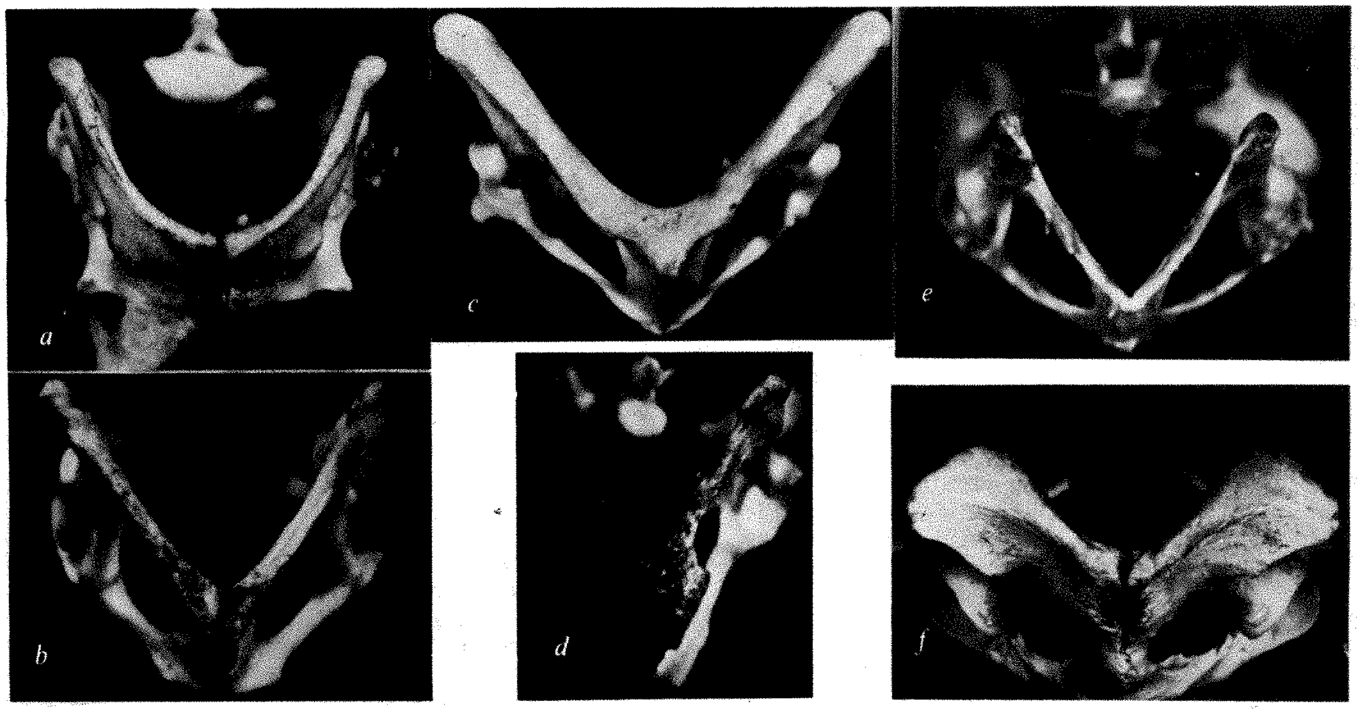

Fig. 2 Ischial arc in posteroventral view in: *a*, *Tachyglossus* (×1.4); *b*, *Didelphis* (×1.75); *c*, *Macropus* (×0.7); *d*, *Barunlestes* (×3.5); *e*, *Tupaia* (×2.8); *f*, *Equus* (×0.28).

arc in modern eutherian mammals varies from comparatively narrow and V-shaped in nidicoles (Fig. 2*e*) up to a widely open condition, forming an obtuse angle in advanced nidifuges (Fig. 2*f*). The differences in shape of the ischial arc between marsupials and nidicolous eutherians are not very great, and in some marsupials (for example, *Macropus*) the ischial arc is more widely open dorsally than in certain nidicolous eutherians (for example, *Tupaia*). In spite of this, one can state that in viviparous mammals in general, the ischial arc widens with prolonged periods of gestation. It follows that the shape of the ischial arc together with other characters (such as the occurrence of marsupial bones) may provide some indication of the mode of reproduction in fossil mammals. In *Barunlestes* the ischial arc is V-shaped and very narrow (Fig. 2*d*). Together with the presence of the marsupial bones this may indicate a short gestation period.

Lillegraven[9,10], partially on the basis of embryological evidence of Müller[11], suggested that the neonate in common ancestors of marsupials and placentals was born free of egg membranes, in an immature, marsupial-like state. It seems possible that Cretaceous eutherian mammals retained this primitive condition and were born in a much more nidicolous state than most modern eutherian mammals. More detailed discussion on the marsupial characters in the skeletons of Cretaceous Eutheria and their phylogenetic implications will be published later.

I thank Dr M. Archer (Queensland Museum, Brisbane) for the loan of marsupial skeletons and for the most valuable information on the structure of the pelvis in Australian marsupials; Professors A. W. Clemens (University of California, Berkeley) and J. A. Lillegraven (California State University, San Diego) for comments and discussion.

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Received April 5; accepted May 5, 1975.

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Comparative effects of atmospheric pollution and cigarette smoking on carboxyhaemoglobin levels in man

ATMOSPHERIC pollution by carbon monoxide (CO), mainly derived from the motor-car, has given rise to worldwide concern in recent years. In an attempt to limit this form of pollution legislation has been introduced, both in the US[1] and Britain[2], to control the level of CO emitted by the exhaust of the internal combustion engine. The cost of such measures would be high, both in terms of engine modification and the inevitably increased fuel consumption. The question arises: What would be the probable effect of such measures on man?

When inhaled, CO combines with haemoglobin in the red cells to form the stable compound carboxyhaemoglobin (COHb) in a quantitative manner, and although the affinity of haemoglobin for CO is over 200 times greater than its affinity for oxygen the rate of combination is markedly slower[3]. The main physiological effects of COHb are twofold; an absolute decrease in haemoglobin available for oxygen carriage and interference with oxygen transport by the remaining available haemoglobin. These effects combine to decrease the oxygen supply to the tissues.

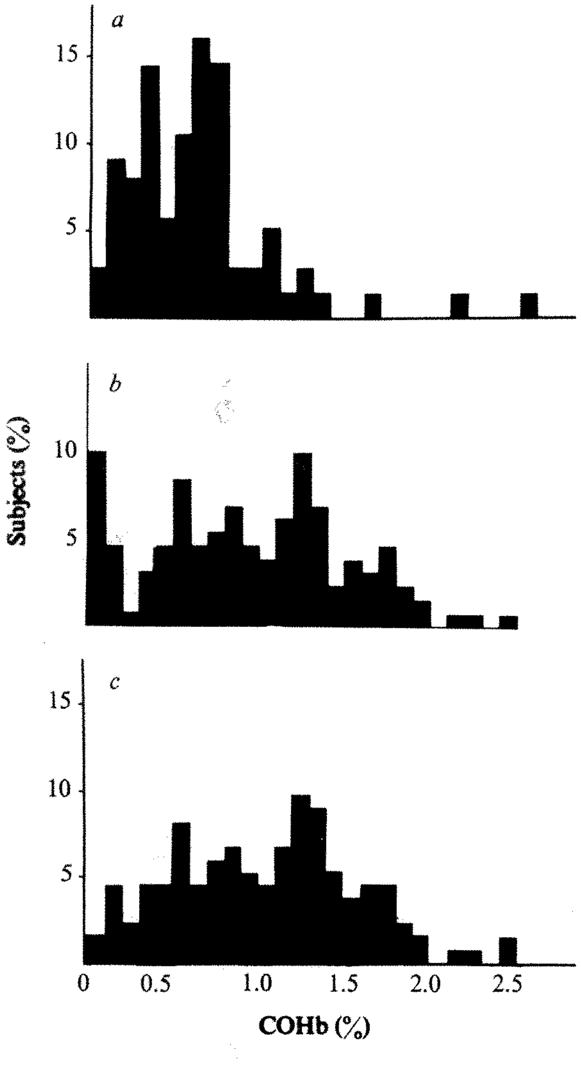
Atmospheric concentrations of CO vary widely throughout the world, ranging from extremely low concentrations in the Central Pacific[4] to over 100 p.p.m. in urban highways in peak conditions of traffic flow[5]. The average concentration in a major city street, however, seems to be about 10–30 p.p.m. and this applies all over Europe[6].

One place near to Britain which has low atmospheric concentrations of CO is the Channel Island of Sark. This is a relatively isolated community where motor cars are forbidden by the Dame of Sark. The only mechanical means of transport on the Island is by diesel powered tractor, which emit only low concentrations of CO. Twenty spot estimations of island air over a 6 d period always revealed concentrations of less than 1 p.p.m. CO. Blood samples taken at the same time were estimated for COHb by the method of Commins and Lawther[7,8]; 78 subjects of both sexes who did not smoke had a mean COHb of 0.68% with a range of 0.2–2.6% (Fig. 1*a*). Female subjects had a mean COHb of 0.70% compared with male subjects whose mean level was lower, 0.61%. This difference is not significant.

In the City of London, although street concentrations of CO are much higher than those in Sark most people are exposed to such an atmosphere for a relatively short period of time. Concentrations of CO in the atmosphere breathed by most people during the greater part of their working lives are in fact much lower than those in the street. By far the greatest proportion of the population commute from suburban areas where atmospheric concentrations of CO are only 1–2 p.p.m. away from streets (unpublished data).

In the Outpatient Department of St Bartholomew's Hospital (where smoking is forbidden), the ambient CO concentrations ranged between 2 and 4 p.p.m. The resultant mean COHb level in 120 non-smokers was 0.97% with a range of 0.2–2.5% (Fig. 1*b*). In an adjacent City office (where smoking was permitted) the ambient concentration was slightly higher, 3–8 p.p.m. CO. COHb levels in 100 office workers (who were non-smokers) were also slightly higher, a mean of 1.12% with a range of 0.1–2.7% (Fig. 1*c*). COHb levels were also estimated at the same time for 100 office workers in the same environment who were regular cigarette smokers. Their mean COHb was 5.5% with a range of 2.2–13.0% (Fig. 2)—very much higher than any of the previous groups.

Fig. 1 COHb levels in three populations of non-smokers. *a*, Island of Sark (the two highest levels were both from women who may have been exposed to a defective butane gas burner before sampling). Mean COHb 0.68% atmospheric CO < 1 p.p.m. *b*, City of London, where smoking was not permitted. Mean COHb 0.97%; atmospheric CO 2–4 p.p.m. *c*, City of London, where smoking was permitted. Mean COHb 1.12%; atmospheric CO 3–8 p.p.m. Groups *b* and *c* were sampled between 1400 and 1600.



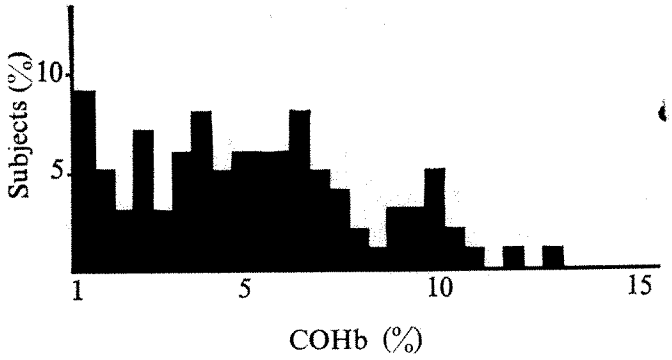


Fig. 2 COHb levels in 100 city office workers who smoked. All samples were taken between 1400 and 1600.

The resting COHb level of a non-smoker, not exposed to atmospheric carbon monoxide is not zero but about 0.7% (ref. 9). This is derived from the endogenous production of CO which is produced in the body by the normal breakdown of haem-containing compounds, mainly haemoglobin. When man is continually exposed to atmospheres similar to those breathed by most workers in the City of London but not contaminated by cigarette smoke his COHb rises on average by about 0.3%. If smoking is permitted in this atmosphere slightly higher concentrations of CO are found and in turn, slightly higher COHb concentrations in those who breathe it—the increase being about half that resulting from the urban environment alone. This increase is almost certainly caused by 'passive smoking'—the inhalation of side-stream and exhaled main-stream smoke from fellow workers who actively smoke[10]. Main-stream cigarette smoke contains up to 5% CO (ref. 11), cigar smoke perhaps even twice as much. Active inhalation of this produces levels of COHb which are considerably higher than those found in any other group of subjects who work in the City of London. Even traffic policemen, who spend long periods of time in busy city streets, have significantly higher COHb levels than non-smoking policemen performing similar duties[12]. It is unlikely that COHb levels similar to those found in our non-smoking subjects, could be shown to be harmful to health. Levels similar to those commonly found in cigarette smokers, however, are probably responsible for many disease processes particularly those associated with the cardiovascular system[13,14].

To conform with the present European standard of exhaust emission control, the cost of the necessary carburettor modification is relatively modest. To conform with projected US standards the overall retail cost of a vehicle would be increased by about 9%. Losses of fuel economy would be of the order of 15% (ref. 15). The annual cost of such legislation could well be many hundreds of millions of pounds. In addition there is a world shortage of fossil fuels. Replacement of London's atmosphere by one similar to that of Sark might be expected to reduce the mean COHb level of its inhabitants by 0.3%. In practice the effects would be even less. Even the most severe controls cannot, at present, remove all the emitted CO from a vehicle exhaust. Furthermore the many other sources of urban atmospheric CO will not be affected. Even if a reduction is achieved, it will only affect a relatively small proportion of the population—dwellers in large towns who do not smoke. The effects on smokers could not even be measured—and 70% of adult males smoke[16].

I thank Professor P. J. Lawther for invaluable criticism and advice, and the late Dame Sibyl Hathaway, OBE, for permission to take samples on the Island of Sark.

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Received March 5; accepted May 21, 1975.

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Heavy metal pollution and mineralisation of nitrogen in forest soils

LITTER decomposition and soil enzymatic activity in forests surrounding metal-processing industries are influenced by high concentrations of deposited heavy metals[1–2]. Very little seems to be known about the effects of heavy metal pollution on nitrogen mineralisation rates in natural soils. Reports on nitrogen mineralisation and nitrification rates after addition of heavy metal salts to mineral soils are not quite unanimous[3–6]. There is evidence, however, that the ammonification rate of some vineyard soils is retarded by accumulated Cu[7]. I have determined the relationship between nitrogen mineralisation rate and the degree of Cu and Zn pollution in conifer forest soils, and found that soil nitrogen mineralisation is impaired by industrial metal pollution. I also conclude that a Cu and Zn concentration of three times the background level is sufficient to bring about a measurable disturbance of the nitrogen mineralisation rate.

I have studied the surroundings of Gusum (58°15′N, 16°33′E), a village (1,500 inhabitants) in south-eastern Sweden, where only one industry is located, a brass mill founded in 1661. Considerable amounts of Cu and Zn, but also some Pb and Cd, have been deposited in the surroundings[2]. Cu and zinc concentrations of the organic topsoil (mor horizon) in sites adjacent to the mill are as high as 10,000–15,000 p.p.m. and 15,000–20,000 p.p.m. dry weight, respectively. Background concentrations (Cu: 12–20 p.p.m., Zn: 100–200 p.p.m.) are found 7–9 km from the village. The vegetation near the mill has been severely injured.

The vegetation in the Gusum area is mainly coniferous woodland, with predominantly acid siliceous soils (archaean moraines), usually podzolic types with a well defined mor horizon. Exposed bedrock (granite and gneiss) is widespread. The area is sparsely inhabited and there are no other industries of importance. As insignificant amounts of other pollutants are emitted from the mill and the village, the influence of the deposited heavy metals on soil biological processes may be studied without severe interference from other pollutants, a rare circumstance in empirical studies on industrial ecology.

Table 1 Relationship between nitrogen mineralisation rate (y) and certain soil variables (x)

Total correlation, r_{xy}		
	log Cu log y	−0.784***
	log Zn log y	−0.734***
	log Pb log y	−0.696***
	log Cd log y	−0.669***
	$p\mathrm{H}_{\mathrm{KCl}}$ log y	0.087
Partial correlation, $r_{x_1y.x_2}$		
	log Cu log y . $p\mathrm{H}_{\mathrm{KCl}}$	−0.797***
	log Cu log y . log Cd	−0.558***
	log Cu log y . log Pb	−0.501***
	log Cu log y . log Zn	−0.461***
	log Pb log y . log Cu	0.001
	log Cd log y . log Cu	0.120
	log Zn log y . log Cu	0.241*
	$p\mathrm{H}_{\mathrm{KCl}}$ log y . log Cu	0.248*

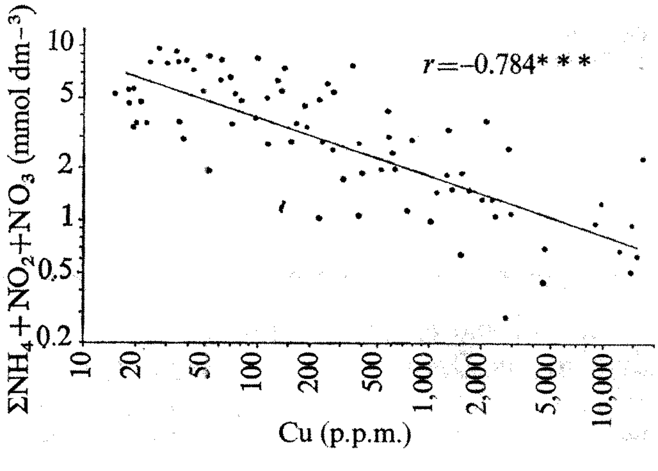
Stepwise regression

Step no.	Variables in equation					Multiple correlation
1					log Cu	0.784
2				$p\mathrm{H}_{\mathrm{KCl}}$	log Cu	0.799
3			log Zn	$p\mathrm{H}_{\mathrm{KCl}}$	log Cu	0.803
4		log Cd	log Zn	$p\mathrm{H}_{\mathrm{KCl}}$	log Cu	0.805
5	log Pb	log Cd	log Zn	$p\mathrm{H}_{\mathrm{KCl}}$	log Cu	0.805

Basis of calculation: gram dry matter ($n=80$). Level of significance: $^{***}P<0.001$, $^{*}P<0.05$.

Field sampling was carried out from March 30–April 2, 1974, on the mor horizon on the northern side of spruces, which grow in closed stands in 80 sites distributed in a stratified random way in all directions within 7 km of the mill. At each site three samples were bored to a depth of 40 mm using a steel cylinder with a sharp edge (volume 154 cm^3). The samples were transferred to plastic containers of a slightly larger volume, thus minimising the risk of disturbing the stratification. The material surrounding the cylinder was collected for chemical determinations. Aerobic incubation of the cylinder samples was carried out in the containers for 10 weeks (humid chamber, 22±0.5 °C, water content 47–50% of the water-holding capacity). The three samples from each site were combined for analysis. Mineral nitrogen was extracted in 1 M KCl for 2 h and an aliquot of the filtrate subjected to a two-step distillation[6]. Ammonia was determined spectrophotometrically by the indophenol method[8]. As the nitrite and nitrate fraction was small in most samples, only the increase during incubation in the sum of ammonium, nitrite and nitrate is reported. Concentrations of total Cu, Zn, Cd and Pb were determined by atomic absorption spectrophotometry after wet digestion of organic matter with HNO_3 and $HClO_4$. $p\mathrm{H}_{0.2\,\mathrm{M\,KCl}}$ was determined electrometrically. The data were subjected to correlation and regression analysis to quantify the relation-

Fig. 1 Relationship between copper concentration and nitrogen mineralisation rate (increase of $\Sigma NH_4+NO_2+NO_3$ during aerobic incubation of mor samples in undisturbed stratification for 10 weeks at 47–50% of the water-holding capacity and 22 °C). Double logarithmic scale.



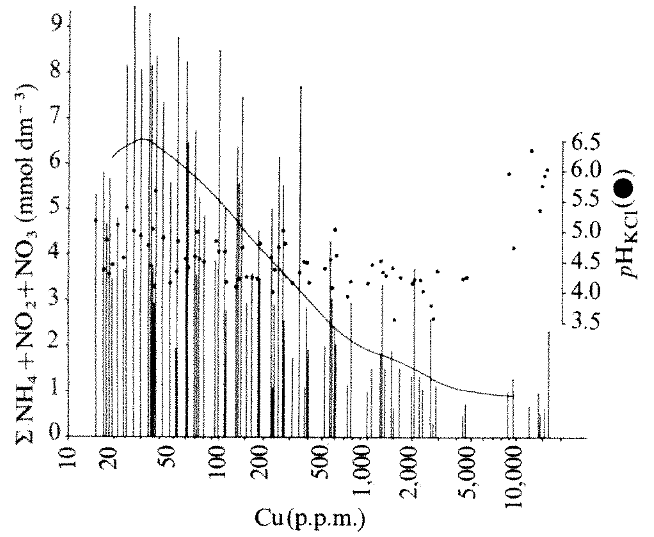


Fig. 2 As Fig. 1 but semilogarithmic scale. Increase in mineral nitrogen indicated by columns and a curve of successive means. ●, pH$_{KCl}$.

ship between heavy metal concentrations and biological variables. A multiple regression program was also tested (BMD O2R, step-wise regression, Health Sciences Computing Facility, UCLA, California).

The increase in mineral nitrogen was calculated as mmol dm^3 considered the ecologically most appropriate basis of calculation in this case. An approximately straight regression line was obtained for increase in mineral nitrogen with Cu concentration (double log scale, Fig. 1). The partial correlation coefficients are highly significant ($P<0.001$) for Cu but not for Zn, Cd or Pb (Table 1). The multiple correlation coefficient is somewhat improved, when pH$_{KCl}$ is included in the equation together with Cu. Inclusion of other variables improves the coefficient very little.

More detailed information on the relationship is obtained from a semilogarithmic presentation (Fig. 2). With increasing degree of metal pollution the amount of nitrogen mineralised is reduced from 5–6 to about 1 mmol dm^{-3}. There are indications of a downward-sloping curve from a Cu concentration of about 50 p.p.m. (corresponding to three times the background level). There is some evidence for a rising curve in the interval 15–30 p.p.m., but this needs to be confirmed. The higher pH$_{KCl}$ in heavily polluted samples is caused by the neutralising effect of the deposited metal dust, possibly also to a lower biological activity.

We conclude that heavy metal pollution, at least of Cu, is a limiting factor for the nitrogen mineralisation rate in acid forest soil. Moderate amounts of pollutants are sufficient to reduce the supply of nitrogen available to plants.

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Genetical and environmental diversity in *Drosophila melanogaster*

NATURAL populations often show extensive genetical diversity for soluble proteins[1]. It is considered by some that much of this variation has no adaptive value[2]. In contrast, others consider that natural selection is responsible for shaping the gene pools of populations[3–4]. Evidence exists for a genetical response to selection through environmental heterogeneity; for example, a positive and almost linear relationship was observed between the extent of the environmental heterogeneity to which laboratory populations of *Drosophila willistonii* were subjected and the average heterozygosity of an individual[5]. More recent work with *D. pseudoobscura* has been interpreted as agreeing with the latter observation[6]. These results are of interest, as in theory, genetical polymorphism can be maintained within populations living in variable environments, although the conditions for a stable equilibrium are restricted[7].

We have tested for and assessed the proportion of loci affected by selection as a result of environmental difference, and compared the effect of uniform and variable environments on average genic heterozygosity. Detailed results and analyses will be published elsewhere. A laboratory population of *D. melanogaster* 'Texas' was used and details of the origin and maintenance of this population have already been described[8]. Genetical variation was observed in Texas at eight out of a total of 22 protein–enzyme loci studied using starch and polyacrylamide gel electrophoresis. The *pink* locus (an eye-colour mutant) was also polymorphic. The average genic heterozygosity per individual in Texas was 8.2%. On average the population size was 3,000 adults.

Six subpopulations of Texas were independently established within 3 weeks. Two replicate subpopulations, both containing 2,000 adults (equal numbers of each sex), were placed into each of the following three environments: (a) the temperature alternated between 18 and 25 °C every 3 d and food medium consisted of oatmeal, agar, molasses, live baker's yeast and sultanas; (b) kept at −15 °C for 30 min every 3 d, otherwise at 25 °C with food medium the same as for (a); (c) kept at 25 °C and food medium consisted of agar, sucrose and dried baker's yeast only. Thus, environments (a) and (b) contain more environmental variables than (c).

Our test of selection was based on genotype frequencies and preliminary investigations showed that two of the nine polymorphic loci in Texas (*Lap-A* and *Pgm*), were not suitable for this study. Activity levels of *Lap-A* were low and it was difficult to score the genotypes from a zymogram. The allele frequencies at the *Pgm* locus were 0.02 and 0.98; for reasons of labour the *Pgm* locus was not studied. Genotypes at the *pink* locus were ascertained from the progeny of backcrosses of flies to a strain homozygous for the pink allele.

Egg samples were taken from all of the experimental populations after 27 weeks (occasion 1), and raised to adults in bottles. The populations were resampled when they were 54 weeks old (occasion 2). The genotype frequencies were determined for all loci on 200 individuals. The *Lap-D* locus was only studied on occasion 2. As the generation time for a large population of *D. melanogaster*, kept at 25 °C and with overlapping generations, is approximately 3 weeks[9], the populations were sampled at generations 9 and 18.

Three of the 39 χ^2_2 values which compare genotype frequencies in replicate populations were statistically significant. In this experiment the following items will contribute to variation between the replicate cages: variation between the samples of eggs used to form the replicate populations; variation in the samples of eggs taken from the populations at the two sampling times; random genetic drift; and when the type of selection differs in two apparently similar replicate cages, for example, the effect of environmental variation which occurs in a temporally different sequence in replicate populations[10].

To determine whether the variation in genotype frequencies was greater between environments than between replicate cages, the following test was carried out: Since by definition a sum of squares (SS) $= \chi^2\sigma^2$ (where σ^2 is a theoretical variance) it follows that an observed variance $V_A = \chi^2{}_A\sigma^2{}_A/\text{d.f.A}$, where d.f.A. are the degrees of freedom of the variance A. For two observed variances V_A and V_B, V_A/V_B is a variance ratio

Table 1 Variance ratio test for environmental selection

Locus	χ^2_4 Between environments		χ^2_6 Replicate cages		Variance ratio for 4 and 6 d.f.	
	Occasion 1	Occasion 2	Occasion 1	Occasion 2	Occasion 1	Occasion 2
Est-6	34.38	13.11	3.65	4.19	14.13†	4.69*
Odh	9.65	11.30	4.73	17.48	3.06‡	0.97‡
Pink	7.92	30.21	3.23	4.35	3.68‡	10.42†
Adh	7.24	22.41	6.95	2.06	1.56‡	16.32†
α Gpdh	21.18	4.26	7.38	4.50	4.30‡	1.42‡
6-Pgdh	18.59	29.87	3.89	9.39	7.17*	4.77*
Lap-D	—	19.09	—	2.59	—	11.06†

*$0.05 > P > 0.01$.
†$0.01 > P > 0.001$.
‡Not significant.

(for which $\sigma^2_A = \sigma^2_B$ is the null hypothesis), and is equal to $(\chi^2_A/\text{d.f.A})/(\chi^2_B/\text{d.f.B})$. The consistency of the replicate cages for each locus, sampled on the two occasions, for each of the three environments was tested by a contingency χ^2 for 2 d.f. χ^2_B for 6 d.f. was obtained by summing these χ^2_2 over the three environments. For both sampling times, the genotype frequencies from replicate cages in the same environment were pooled, and χ^2_A for d.f. calculated as a test of the similarity of the genotypic distributions in the three environments.

Where there are no significant differences between replicate cages, $\chi_A{}^2$ is a valid and a more powerful test of selection, but if replicate cages do differ in genotype frequency then we must use the variance ratio test; we have no test for the fourth type of selection described above. Although the $\chi_A{}^2$ values are shown in Table 1, we have discussed our results using the variance ratio as a test for selection. This will tend to underestimate the importance of selection when there are no significant differences between replicate cages. The variance ratios are shown in Table 1, and it is seen that by occasion 2, the environments have differentially affected the genotype frequencies at all loci except *Odh* and *αGpdh*.

This is only one measure of selection. Selection may be difficult to detect if the genotype frequencies show a similar or unidirectional responses in the three environments. As the population sizes were relatively large compared with the number of generations which lapsed between occasions 1 and 2, any effect of random genetic drift will be negligible. A difference between the genotypic distributions at the two times of sampling would be additional evidence of selection. Such a test has been carried out by the variance ratio test described earlier, and the details are shown in Table 2. There is now evidence for selection at the *Odh* and *α Gpdh*; selection in one form or another has resulted in changes of genotype frequency at all seven loci.

The percentage heterozygosity at all loci on occasion 2 (Table 3) was transformed into angles. Analysis of variance of the transformed data showed that the variation between replicate cages was not greater than the theoretical variance σ^2, calculated as 820.7/200. The three environments did not differ in their average level of heterozygosity (calculated over the seven loci) but the individual loci responded dissimilarly to the three environments. McDonald and Ayala's[6] results, although not as striking as those of Powell[7], showed an increase in the average genic heterozygosity of individuals from environments that varied in one or two levels of heterogeneity. In contrast, our experiments have shown that individuals from populations maintained in the most variable environments were not, on average, the most heterozygous. There is no *a priori* guarantee in experiments of this type, of the way in which the genetical variation will respond to the applied environmental variation. It may well be that our variable environments did not contain sufficiently effective environmental heterogeneity to bring about such results as were obtained by Powell.

We have shown, however, that the environments were sufficient to change the genotype frequencies at all of the observed loci. Analysis of variance of McDonald and Ayala's

Table 2 Variance ratio test for selection between the two sampling times

Locus	Environment	χ^2_2 Between occasions	χ^2_4 Replicate cages	Variance ratio for 2 and 4 d.f.
Est-6	a	5.11	3.56	2.87§
	b	0.91	1.12	1.63§
	c	48.45	3.76	25.77†
Odh	a	5.45	14.60	0.75§
	b	14.80	2.06	14.37*
	c	2.75	5.55	0.99§
Pink	a	3.09	3.06	2.02§
	b	1.03	3.50	0.59§
	c	1.13	2.01	1.12§
Adh	a	10.46	2.26	9.26*
	b	6.06	5.78	2.09§
	c	2.95	1.06	5.57§
α Gpdh	a	28.27	1.92	29.45†
	b	3.49	0.72	9.70*
	c	14.91	9.25	3.22§
6-Pgdh	a	9.50	7.50	2.53§
	b	8.62	1.60	10.78*
	c	27.29	1.54	35.45‡

*$0.05 > P > 0.01$
†$0.01 > P > 0.001$
‡$P < 0.001$
§Not significant

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Table 3 Percentage heterozygosity

Environment	Replicate	*Est-6*	*Odh*	*Pink*	*Adh*	α *Gpdh*	*6-Pgdh*	*Lap-D*	Average percentage heterozygosity per individual
a	1	32.9	29.8	25.8	15.6	23.6	26.3	34.8	7.55
	2	38.0	16.7	22.5	18.7	20.5	35.7	35.3	7.49
b	1	41.0	20.5	20.9	25.2	23.1	32.0	33.2	7.84
	2	43.4	15.5	27.0	23.9	24.7	29.2	35.2	7.95
c	1	45.2	20.0	16.3	19.7	30.1	20.2	25.4	7.07
	2	46.5	23.6	17.0	16.6	27.8	15.3	21.3	6.93

data after angular transformation showed, as we have done, a strong interaction between loci and the level of environmental heterogeneity. Such an interaction is again evidence for selection. The variation between environments in percentage heterozygosity was also calculated for individual loci from McDonald and Ayala's data. A χ^2 test for selection was calculated as SS/σ^2, and eight out of the twenty tests were statistically significant.

Nevertheless, these results do not exclude the possiblity that selection is acting on closely linked loci, rather than the observed loci themselves. We have tried to minimise the effect of linkage. The Texas population has been maintained in the laboratory for over 200 generations. Any non-random association between alleles caused by any initial 'founder effect' will be confined to extremely closely linked loci. Also each of the subpopulations was started with 2,000 flies. Chromosomal inversions represent extreme cases of linkage. Associations between enzyme polymorphisms and chromosomal inversions have been described in natural populations of *D. pseudoobscura*[11] and *D. melanogaster*[12,13]. In *D. willistonii*, however, similar associations were not detected[14]. In the Texas population all of the possible combinations of alleles from linked loci can be extracted, and limited evidence has shown no evidence of inversions relevant to our study. All of the loci studied have shown evidence of selection. It is unlikely that every case of observed selection can be explained as being caused by selection at another closely linked locus.

In nature, environmental diversity is on a greater scale than that which we have created in the laboratory. As such, selection must account, directly or indirectly, for the behaviour of a substantial fraction of the genetical diversity within populations.

A. M. acknowledges the support of a scholarship from Makerere University, Kampala.

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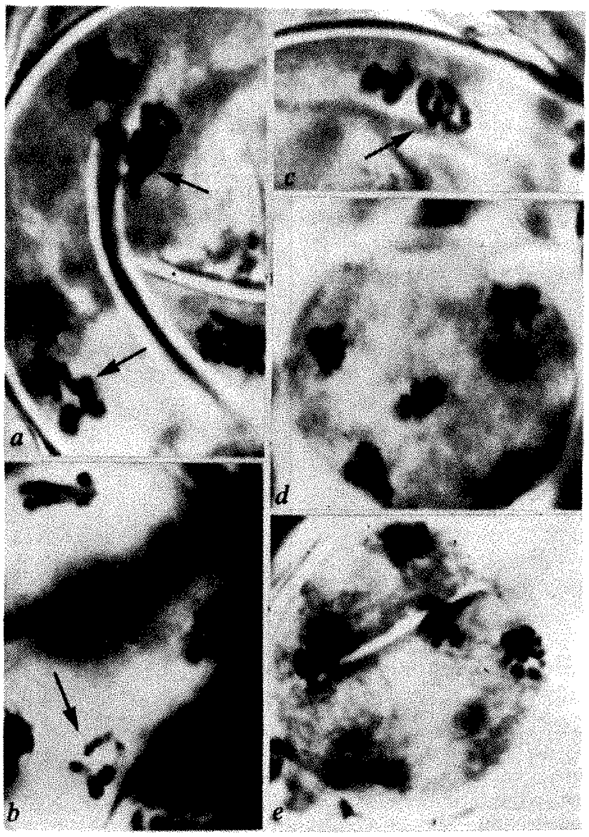
Chromosome size differences in *Phytophthora palmivora*, a pathogen of cocoa

Phytophthora palmivora is one of the most ubiquitous and destructive plant pathogens of tropical tree crops such as palms, cocoa, citrus and rubber[1]. On cocoa, where it causes black-pod disease, annual losses are conservatively estimated at 10% of the crop[2]. Progress in disease control has been impeded by confusion over the taxonomy of the species, which has become a pigeon-hole for a number of structurally similar *Phytophthora*. This situation has been compounded by preoccupation with compatibility types, whereas morphological differences have been neglected[3].

Two isolates of *P. palmivora* from cocoa in Nigeria, P131 and P132, were obtained from the Commonwealth Mycological Institute; P131 was found to be of the A2 mating or compatibility type and P132 of the A1. When mated together on carrot agar and examined cytologically, they were found to have distinctive nuclei in their sex organs. One, the large (L) chromosome type had a basic number of 5 or 6 comparatively large chromosomes at metaphase, with a multiple association of at least four chromosomes (Fig. 1*a–c*). The other, the small (S) chromosome type had a basic number of 9–12 much smaller chromosomes (Fig. 1*d* and *e*). On pairing P132 with an A2 isolate of *P. drechsleri* P6503 (ref. 4) it was confirmed that P132 was the L chromosome type and therefore P131 the S type.

Further cocoa isolates from Cameroun, Nigeria and

Fig. 1 *a–c*, Metaphase I configurations from oogonia of the L type, showing multiple associations (arrowed). *d* and *e*, Metaphase I configurations from oogonia of the S type. Aceto-orcein squash method. × ~ 2,200.



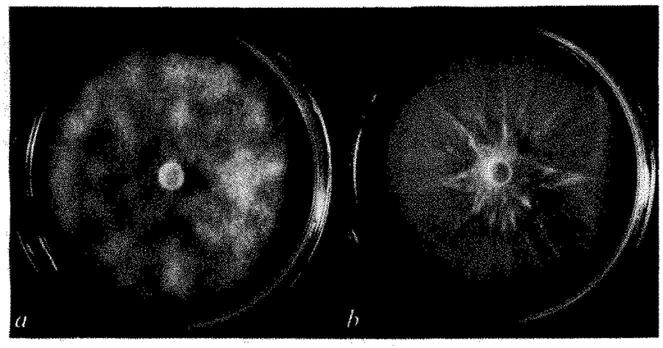


Fig. 2 Cultures of *Phytophthora* isolates from cocoa. *a*, Typical L type from Nigeria; *b*, typical S type from Ghana. Grown on carrot agar in darkness at 25 °C.

Table 2 Distribution of S and L types among *Phytophthora* isolates from cocoa in West Africa, based on chromosome structure and culture morphology

	No. of isolates*			
	L type		S type	
	A1	A2	A1	A2
Cameroun	1 (1)	1 (1)	—	2 (2)
Nigeria	430 (3)	—	—	11 (5)
Togo	—	—	—	2 (0)
Ghana	—	—	—	7 (3)

* Figures in parentheses denote number of isolates examined for chromosome structure.

Ghana have been examined in Britain for mating type and chromosome structure. Of ten A2 isolates examined cytologically, nine were of the S and one (from Cameroun) of the L chromosome type (the latter confirmed by pairing with an A1 isolate of *P. drechsleri* P6500; ref. 4). All five A1 isolates examined were of the L type. These results establish the existence of two chromosome types of *P. palmivora* on cocoa in West Africa. They also demonstrate that both A1 and A2 compatibility types are present in the L type.

Table 1 Comparison of large and small chromosome types of *P. palmivora*

Chromosome type	Growth rate on carrot agar (mm d^{-1})*	Growth in Amazon cocoa pods: lesion size (cm)†	Sporangial dimensions (μm)‡	
			Length	Length–breadth ratio
Large (L) (n = 5–6)	7.4 – 9.2	12.9 – 15.9	34.0 – 38.1	1.34 – 1.47
Small (S) (n = 9–12)	9.2 – 12.2	16.9 – 21.4	41.1 – 45.7	1.67 – 1.93

* Mean range of ten L and ten S isolates over 3 d at 25 °C in darkness.
† Lesion size, length plus breadth; mean range of nine L and nine S isolates, five replicates per isolate, after 96 h at 23 °C in the laboratory.
‡ Sporangia from cocoa-pod lesions, mounted in distilled water. Mean range of ten L and ten S isolates, 100 measurements per isolate.

L and S types could be distinguished on carrot agar, the L types (both A1 and A2) producing plentiful woolly aerial mycelium (Fig. 2*a*) and the S types growing faster (Table 1) and producing much less aerial mycelium with a distinctive stellate pattern in the centre of the colony (Fig. 2*b*). When inoculated into cocoa pods in the laboratory, L types formed a diffuse-edged surface lesion, and S types a faster developing sharp-edged lesion (Table 1). On pods the L type also formed shorter and more rounded sporangia than the S type (Table 1).

These comparative studies show that the S and L chromosome types correspond to the cocoa and rubber groups of *P. palmivora* described by Ashby[5], the typical and atypical groups of Tucker[6], the 'G' and 'N' forms of Turner[7], and morphological forms 1 and 2 of Waterhouse[3] respectively. The difference in chromosome size and number between the S and L types show that they must now be regarded as distinct species (details will be published elsewhere).

The occurrence of two species of *Phytophthora* on cocoa has considerable implications for the control of black-pod disease. A preliminary survey carried out to establish the distribution of the L and S types in West Africa has shown that the L type (A1) is the principal disease-causing organism in Nigeria (Table 2), the S type (A2), with one exception, having been isolated only from soil or rotting husks beneath cocoa trees. In contrast the survey results, together with Turner's morphological studies[7], indicate that the S type (A2) is the only one associated with black-pod disease in Ghana. Behavioural differences between the two species (Table 1) may account for differences between the disease situations in Nigeria and Ghana, and should be taken into account in the application of chemical control measures and in the breeding of cocoa for disease resistance. Preliminary field observations already indicate significant pathological differences between the two species (M.J.G. and A. C. Maddison, unpublished).

The occurrence of sexually compatible L and S types on cocoa in Nigeria and elsewhere raises the possibility of hybridisation between them, and of the occurrence of intermediates. So far hybrids have not been identified. It is likely that they would have reduced viability and that only selfed oospores would survive in nature.

We thank Drs A. C. Maddison and B. Boccas for some of the *Phytophthora* isolates.

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Received April 1; accepted May 2, 1975.

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Direct magnetic separation of red cells from whole blood

THE magnetic properties of haemoglobin are well established[1]. The prosthetic group of haemoglobin has a protoporphyrin structure (ferrohaem) in which the iron atom is ionically bound. Consequently there are in deoxyhaemoglobin four unpaired electrons per iron atom leading to a paramagnetic moment of 5.35 Bohr magnetons per haem group. Oxyhaemoglobin on the other hand is diamagnetic. Other iron-containing haemoproteins such as ferrihaemoglobin have been shown by magnetic susceptibility measurements[2] to possess magnetic moments per haem group which correspond reasonably closely to those predicted from the number of unpaired electrons per iron atom. Consequently red blood cells containing deoxyhaemoglobin when placed in a magnetic field B_0 (Tesla) with field gradient dB_0/dz may be expected to experience a magnetic force F_M (Newtons) in the z direction given by

$$F_M = (\chi V/\mu_0)B_0(dB_0/dz) \qquad (1)$$

where χ is the (SI) susceptibility of the red blood cell, V (m^3) its volume and μ_0 the permeability of free space.

In spite of this known property the only previous recorded attempt by Levine[3] to increase the sedimentation rate of red cells in whole blood by placing them in a magnetic field gradient was unsuccessful. According to our own estimates this null result is not surprising as for the low field gradient operative in Levine's experiment (approximately 0.1 T m^{-1}) the force predicted by equation (1) is only a few per cent of the gravitational force experienced by the red cells. The development of a new type of high gradient magnetic separator, as described by Oberteuffer[4], which enables magnetic field gradients of the order of 10^4–10^5 T m^{-1} to be achieved has led us to reassess the possibility of the magnetic separation of blood components. We believe that we have demonstrated that HGMS can be used an as effective means of separating red cells from whole blood.

In the present experiment a polyethylene cylinder of 60 ml capacity with inlet and outflow arrangement was mounted between the pole-pieces of a conventional electromagnet. Into this cylinder smooth stainless steel wire of circular cross section and 25 μm diameter was randomly packed to a volume of 40 ml. Approximately 2% by volume of this 40 ml 'filter' was actually occupied by wire. The length of the filter was less than the diameter of the magnet pole pieces ensuring that the whole wire volume was exposed to a uniform magnetic field. A standard magnetic field of 1.75 T (17.5 kgauss) was used and the magnetic field gradient close to the wires was estimated to be 8×10^3 T m^{-1}. Isotonic sodium dithionite ($Na_2S_2O_4$) was used both as a reducing agent and as a carrier for the whole blood.

The sample used was whole human blood with ACD anticoagulant in which most of the plasma had been replaced by $Na_2S_2O_4$ solution. The haemoglobin concentration of this standard sample was 11.8 g dl^{-1}. The column was first flushed out with $Na_2S_2O_4$ solution and then with the magnetic field of 1.75 T switched on, 5 ml of the blood sample was applied to the top of the column. This was followed by 270 ml of $Na_2S_2O_4$ solution, introduced slowly to maintain the flow rate through the column at approximately 4.5 ml min^{-1}. From the point when the blood was introduced samples flowing from the column were taken as a function of time. After a certain amount of strong discoloration with red cells or haemoglobin the effluent developed an almost transparent pale pink colour. After approximately 175 ml of solution had passed through the column the magnetic field was reduced to zero and successive 5 ml aliquots were taken. The effluent was observed to change markedly to a deep red colour as the field was switched off. All aliquots were spun to check for cell lysis and then analysed for haemoglobin content.

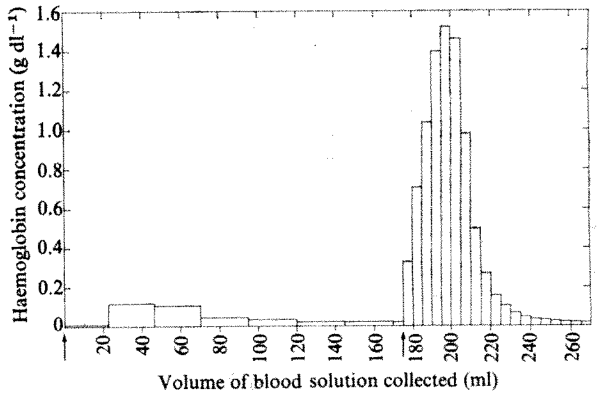

Fig. 1 Haemoglobin concentration of samples as a function of volume flowing through the magnetic separator. First arrow, field on, blood applied; second arrow, field off.

Figure 1 shows the measured haemoglobin concentration of the effluent as a function of time throughout the whole experiment. It can be seen that approximately 70% of the red cells applied to the column were retained until the field was removed. (This implies that 1.30×10^{10} red blood cells were held in the filter.)

Studies of the aliquots showed no evidence in the form of free haemoglobin for immediate red cell destruction. This test was of particular interest as preliminary experiments with 'household' steel wool as the filter medium, although producing similar separation effects, led to a large number of ruptured or severely damaged red cells. The probable reason is that this material possesses an extremely irregular and non-uniform surface.

Scanning electron microscope studies of the cells filtered using the regular stainless steel wire indicated that a small proportion possessed slight morphological abnormalities, mostly in the form of small surface protruberances or indentations. We have yet to determine whether such defects affect the ultimate viability of the red blood cells.

An estimate of the surface area of the filter medium indicates that the wire was completely covered with red cells during the time that the magnetic field was applied. This conclusion is supported by the constant 'hold-back' value of 0.06 g haemoglobin per g wire observed in a number of experiments.

In magnetic separation terms the flow velocity used in the present experiment is extremely low (~10^{-4} m s^{-1}) and further work is necessary to determine how the filter performance varies with flow rate and applied magnetic field.

The successful outcome of this preliminary experiment indicates that high gradient magnetic separators may be used to produce either blood plasma with a low red cell population, or a suspension of red cells free from other blood contents. It also suggests that other haemoproteins in suitable form may be separated in the same way. The application of this principle to specific research or clinical applications is of interest for further investigation.

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Received March 20; accepted May 14, 1975.

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Study of cell development using derepressed mutations

CURRENTLY there is considerable interest in determining how genes are expressed in a highly ordered sequence during morphogenesis. The main genetic approach to this problem has been to study mutants blocked in development. Such mutants are, however, highly pleiotropic; no biochemical or morphological event normally occurring after a point of mutational block is expressed[1]. This, coupled with inadequate knowledge of biochemical events specific to development[2], and the absence of detectable cross feeding between defective mutants[3], makes it difficult to determine the precise lesion in any mutant and its place in control of the sequence. Another method, selecting mutations affecting known biochemical functions can be more rewarding but is also hampered by ignorance of biochemical events relevant to development[2,4].

In these circumstances a useful beginning to genetic study of developmental control is to isolate mutants which undergo development in conditions in which the wild type does not. Such derepressed mutants will be altered in the initiation of morphogenesis. As we are dealing here with microbial sporulation, the term initiation is used for the complex of metabolic and genetic events necessary to set sporulation in train.

Derepressed mutants are important because they are probably defective in control functions, and indicate the number and nature of elements involved in initiation. Moreover, they provide strains from which to select further mutations affecting initiation and the first irreversible steps of the sporulation sequence. By genetic analysis of the interaction between these mutations (for example tests of dominance, position effects and mapping) it is possible to make sensible statements about control. Progress in the genetics of later control elements in the morphogenetic sequence may also be made by judicious mutant selection.

Yeast sporulation is a suitable system in which to select such mutants. Yeast is a simple eukaryote amenable to genetic study and culture conditions leading to initiation of sporulation are known. As in many other microorganisms these are similar to those leading to relief of catabolite repression by both carbon and nitrogen sources[5–7].

Completely derepressed sporulation mutants must be conditional, as once initiated to sporulation a cell is incapable of contributing to population growth[8]. To date two mutants (ID16D and ID19D) which do not sporulate during vegetative growth on YEPD medium (1% yeast extract, 2% peptone, 2% glucose), but unlike the wild type form ascospores at high frequency during starvation in this medium, have been isolated from the homothallic diploid *Saccharomyces cerevisiae* strain S41 (Fig. 1). Mutation to this derepressed phenotype is a rare event occurring at a frequency of less than 1 in 10^8 survivors after ultraviolet mutagenesis. Study of conditions affecting sporulation in both wild type and mutant strains showed that both mutants had lost nitrogen-source repression, but were still subject to glucose repression. In a minimal medium containing glucose and $(NH_4)_2SO_4$, both mutants sporulated in the presence of excess NH_4^+, but sporulation was repressed by excess glucose (Table 1).

Mutants no longer subject to nitrogen repression should sporulate extensively on media in which glucose is replaced by poorer, non-fermentable carbon sources. This was indeed the case for both mutants; on media containing glycerol sporulation was so rapid and extensive that they grew poorly whereas the wild type strain grew normally and sporulated poorly (Fig. 2).

Table 1 Sporulation of strains 1D16D and S41 in minimal media

Culture medium*		Strain	Sporulation† (% asci)	Turbidity† (600 nm)
Glucose conc. (mg ml^{-1})	$(NH_4)_2SO_4$ conc.			
0.5	10	1D16D	30	0.75
		S41	0	0.76
1	10	1D16D	30	1.5
		S41	0	1.4
5	10	1D16D	10	4.6
		S41	0	3.7
10	10	1D16D	0	5.1
		S41	0	3.8
10	0.05	1D16D	0.5	1.2
		S41	0.1	1.1
10	0.1	1D16D	0	2.5
		S41	0	2.5
10	0.5	1D16D	0	5.1
		S41	0	3.8
10	1	1D16D	0	5.2
		S41	0	3.7
Galactose MIN‡		1D16D	15	0.4
		S41	0	3.7

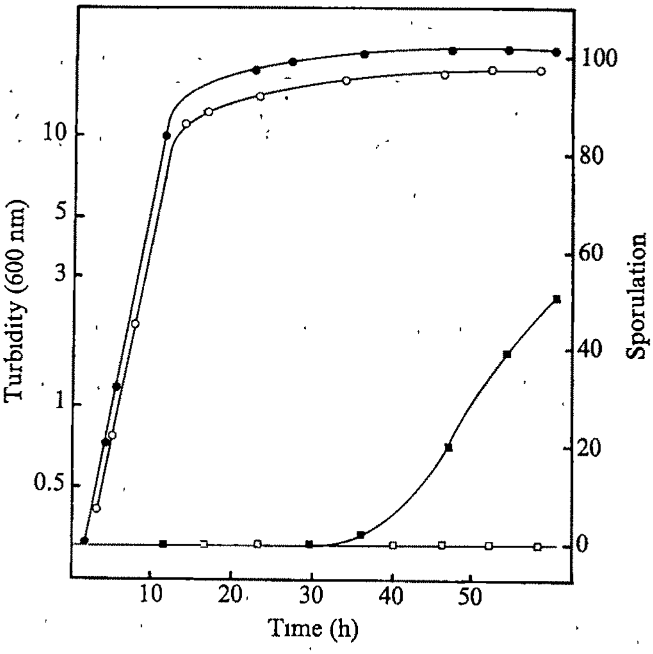
*Cultures were pregrown in YEPD, washed in minimal salts and inoculated at turbidity 0.1 into minimal medium containing: Wickerham minimal salts; arginine at 25 mg ml^{-1} and stated concentrations of glucose and $(NH_4)_2SO_4$.

†% Sporulation and turbidity were estimated after 5 d incubation at 30°C.

‡Galactose minimal medium contained salts and arginine as before, galactose at 20 mg ml^{-1} and $(NH_4)_2SO_4$ at 1 mg ml^{-1}.

It is unlikely that this effect is caused by a mutation affecting solely glycerol metabolism as similar results have been obtained on replacing glucose with other carbon sources (including galactose and acetate) causing less repression. This mutation cannot be dismissed simply as causing a

Fig. 1 Growth and sporulation of wild type and derepressed strain 1D16D on YEPD medium. Wild type strain S41 a/α D/D arg 4-1/arg 4-1 cyh-1 cyh-1 spores were mutagenised according to standard methods[10]. Spores were germinated in YEPD and incubated for approximately 1 week at 34 °C. Cultures were treated with ether as described previously[11] and resuspended in YEPD to allow germination of viable spores. This extended incubation followed by ether treatment was repeated and derepressed mutants were recovered by plating survivors of the final ether treatment on YEPD. S41 is homothallic and allows recovery of recessive mutations expressed only in diploids[10]. Growth was determined turbidometrically after inoculating cultures to turbidity 0.1 from an exponential YEPD culture. Sporulation was estimated by direct counting of percentage asci. ○, Growth of S41; ●, growth 1D16D; □, sporulation of S41; ■, sporulation of 1D16D.



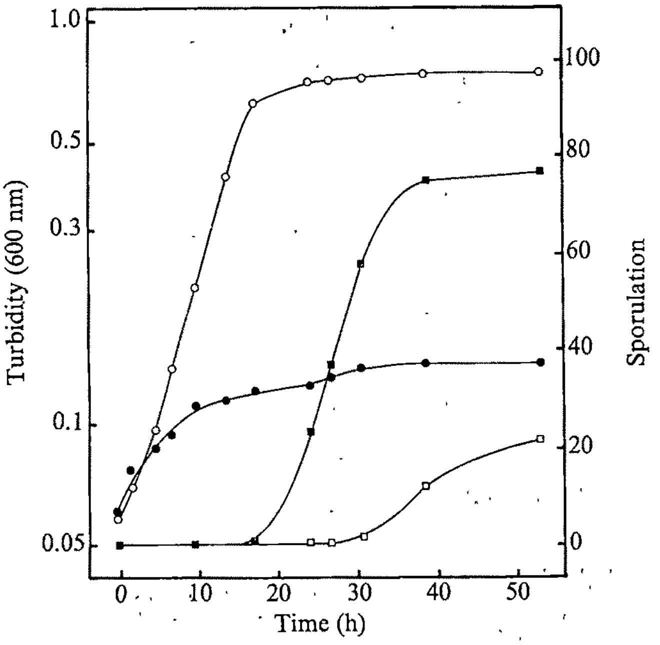


Fig. 2 Growth and sporulation of wild-type and derepressed strain ID16D on glycerol-containing medium. Growth and sporulation (see legend Fig. 1) were estimated on cultures inoculated from YEPD into a fivefold dilution of YEPG medium (1% yeast extract, 2% peptone, 3% v/v glycerol). ○, Growth of S41; ●, growth of ID16D; □, sporulation of S41; ■, sporulation of ID16D.

defect in general cellular metabolism and irrelevant to initiation of sporulation. First, sporulation is not an automatic response to the cessation of growth; the conditions leading to normal sporulation are quite specific and auxotrophic mutants do not sporulate in the absence of the requirement[6]. Secondly, the mutation does lead to sporulation in conditions where the wild type does not sporulate and the effect must therefore be associated in some way with initiation. It may be that this connection is relatively indirect, although reversion results discussed later indicate a fairly direct effect of the lesion. Finally, regardless of the nature of the primary lesion, mutants of this type are important as they are valuable tools for selecting second-site mutations affecting sporulation initiation. This is very useful as mutation to derepression is a fairly rare event.

It is clear that nitrogen-source repression of yeast sporulation can be separated from that due to a carbon source, and there seem therefore to be at least two distinct systems (other than mating-locus control) repressing sporulation It is also likely that completely derepressed sporulation will be the result of at least two mutations. The mutants described here are ideal for trying to isolate the completely derepressed phenotype and also to test the hypothesis that sporulation in yeast is repressed by the same mechanism(s) as synthesis of inducible enzymes in carbon and nitrogen-source repression.

Genetic study has shown that for each mutant nitrogen derepressed sporulation is the result of a single, nuclear mutation. The mutations are recessive to wild type in both sporulation and ability to grow on YEPG. They fail to complement each other, although recombination between the two has been detected at low frequency. The mutations therefore probably represent alleles of the same gene and are designated *spd* 1-1 and *spd* 1-2 for strains ID16D and ID19D, respectively. The *spd* 1 mutation is not linked to the mating-type locus.

Strain ID16D grows so poorly on YEPG that revertants can be isolated easily; the diploid strain reverts spontaneously, or after treatment with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. The majority of revertants tested (69 out of 80) were also revertant to wild type or pseudo wild type sporulation (that is, sporulate on potassium acetate media, but not after starvation on YEPD) Of the other growth revertants, five were asporogenous and six were particularly interesting in that they showed wild type repression at 20 °C, but were asporogenous at 34 °C.

The reversion results are important. First, the coreversion of the two characters, derepressed sporulation and inability to grow on non-repressing carbon sources, indicates that these are interrelated effects of the same mutation. This has been confirmed by meiotic analysis. Secondly, by selecting for revertants which can grow in conditions favouring derepressed sporulation (that is, on YEPG) we have found that in most, if not all, cases the reversion event altered only the ability of the strain to be initiated to sporulate, and not its capacity to complete spore formation in conditions of complete derepression (on potassium acetate). This is a special case confirming our previous prediction that only those organisms defective in initiation of sporulaton will have selective advantage for growth under conditions favouring sporulation rather than growth[9]. This means that reversion of *spd* strains, and competitive growth selection of sporulation-defective mutants from the wild type, are two methods of rapidly obtaining a wide range of mutations in all elements of the initiation/repression mechanism.

The revertants for growth on glycerol which were simultaneously temperature-sensitive for sporulation are particularly interesting as these may be defective in a function (or functions) specific to the initiation of sporulation.

In summary, mutants defective in nitrogen-source repression of yeast sporulation have been isolated and used to study mechanisms initiating sporulation. Sporulation is repressed by at least two separate systems, one caused by the carbon substrate, the other by the nitrogen source. Nitrogen derepressed mutants (*spd* 1) are unable to grow on media containing a poor carbon source and are useful strains in which to select further mutations affecting initiation, and eventually, the first irreversible step(s) of sporulation. These results are the first in an approach to studying genetic regulation of development by selecting mutants undergoing morphogenesis when the wild type does not; in other words, beginning at the beginning.

This research was supported by the SRC I thank I. D Hardie for assistance.

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Received March 6; accepted May 16, 1975.

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Cytotoxicity of isolated plasma membranes from lymph node cells

DESTRUCTION of certain other cells on contact is a specialised capability of some cells of the lymphoid system. Our recent study has indicated that the actual lysis results from osmotic effects[1], but the biochemical process which precedes this step is unknown[2]. If the effector cells are killed before they come into contact with the target cells, cytotoxicity is abolished[2]. Once contact of effector cells and target cells is established, however, killing of the effector cells with specific antibody and complement does not interrupt the lysis of target cells, which continues to completion[3,4]. These observations suggest that viable lymphocytes are required to establish the correct type of contact with target cells (probably because lymphocyte motility is involved[2,5,6]), but that once effective contact has occurred lymphocyte viability is no longer necessary. Since contact of plasma membranes of lymphocytes with those of target cells is required for the lytic process[2], we have examined the possibility that the plasma membranes of lymphoid cells themselves have cytolytic capacity. This interpretation would be consistent with our previous report[7] that subcellular sedimentable fractions from livers of mice infected with *Mycobacterium tuberculosis* (BCG), and heavily infiltrated with mononuclear cells, are lytic for tumour cells.

Lymph node cells were obtained from mice 4 d after contact sensitisation with oxazolone. Such contact sensitisation provides a powerful stimulus for early blast transformation and mitosis of T-lymphocytes (maximum 3-4 d) as well as some later stimulation of B-lymphocytes (maximum 5-8 d)[8]. As shown previously[9] and in Table 1, lymph node cells taken from mice sensitised with oxazolone are able to lyse P-815-X2 mastocytoma cells, especially in the presence of phytohaemagglutinin (PHA).

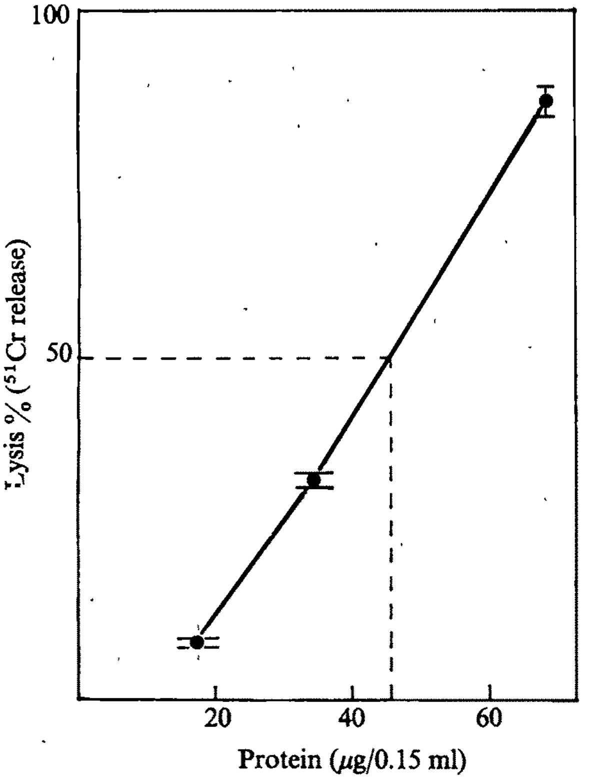
Table 1 Cytolytic activity of activated lymph node cells

Ratio of lymph node cells to mastocytoma cells	PHA	% Lysis of mastocytoma cells
50:1	+	61.2±1.3
50:1	−	7.9±0.4
30:1	+	40.5±0.7
30:1	−	6.3±0.2

Lymph node cells were obtained from the regional lymph nodes of CBA mice which had been skin-sensitised with 4-ethoxymethylene-2-phenyl oxazolone (BDH) 4 d earlier[8]. Lymph node cells were incubated with 10^5 ^{51}Cr labelled P-815-X2 mouse mastocytoma cells in 1 ml Eagle's medium with 5% foetal bovine serum in duplicate tubes for 6 h at 37 °C in the presence or absence of 2 µg PHA (Wellcome). Lysis of mastocytoma cells was assessed by the percentage of ^{51}Cr released from the cells as a proportion of that released by freezing and thawing and with the label released in the absence of lymph node cells subtracted[2].

In further experiments lymph node cells were broken by shearing in controlled conditions[10]. Fractions considerably enriched in plasma membranes were obtained by sucrose-density-gradient centrifugation. As a marker for plasma membrane 5′-nucleotidase and as a marker for lysosomal membranes β-glucuronidase were used[11]. Lysis of P-815-X2 cells was measured by ^{51}Cr release[2]. For each fraction a concentration–response curve was established (Fig. 1), and the relative cytolytic protencies of fractions were expressed as the reciprocal of the protein concentrations required for 50% lysis of target cells after 6 h of incubation. Purified plasma membrane fractions of lymph node cells had considerable cytolytic activity (Figs 2 and 3). In all experiments cytolytic activity was maximal in low density fractions expected to contain plasma membranes[14] and was positively correlated with 5′-nucleotidase activity (Fig. 3) but negatively correlated with β-glucuronidase activity.

Fig. 1 Concentration–response curve for lysis of P-815-X2 mastocytoma cells by a plasma membrane enriched fraction from lymph node cells collected at the 39/36% interface of a sucrose density gradient (see Fig. 2). To 2×10^4 ^{51}Cr labelled mastocytoma cells (see Table 1) in 0.1 ml of Eagle's medium containing 3.5% foetal bovine serum in plastic tubes, twofold dilution of the fraction suspended in 0.05 ml saline was added and the mixtures incubated at 37 °C with occasional shaking for 6 h. Then 0.2 ml of medium was added, the cells centrifuged and 0.2 ml of supernatant taken for counting of radioactivity. The concentration of protein in a fraction required to lyse 50% of the cells was determined by interpolation. The protein content was measured by the method of Lowry using bovine serum albumin as standard. In this example 46 µg protein was required for 50% lysis, and cytolytic activity of the fractions was expressed as the reciprocal (100/46).



These results show that plasma membranes of stimulated lymph node cells have considerable cytolytic potential, much greater than exerted by lysosomal or other fractions. The cytotoxicity of purified plasma membrane preparations contrasts with the lack of such activity in whole homogenates. The purified plasma membranes, probably in the form of microvesicles, are able to diffuse freely and establish contact with the plasma membranes of target cells, whereas in homogenates or killed cells the plasma membranes are entrapped in a gelatinous mass comprising cytoplasmic and nuclear contents. An approximate indication of the efficiency of the plasma membranes in killing target cells can be obtained by comparing the 5′-nucleotidase activity in purified plasma membrane fractions required for lysis of 50% target cells in 6 hours (37–52 nmol Pi per h per ≈ 10 µg protein) with the concentration of this enzyme in the number of viable lymph node cells which have the same cytolytic activity (8–20 nmol Pi per h per ≈ 10^6 cells) in the presence of PHA. The motility of the intact cells would increase the chances of establishing effective contact with target cells and possibly some of the membranes, being "inside out" would have diminished cytolytic activity. Nevertheless, the remarkable coincidence of cytolytic activities of whole cells and plasma membranes observed suggests that the plasma membranes may act as mediators of the cytolytic effects of lymph node cells.

Other proposed mechanisms of cell mediated cytotoxicity are lymphotoxin, and the possible action of lysosomal enzymes. Lymphotoxin is a soluble cytotoxic product of stimulated lymphocytes and macrophages[15,16], the reported rate of action of which is much slower than the cytotoxicity produced by our plasma membrane preparations. Our method of preparation of membranes should eliminate highly soluble lymphotoxin from the system. Evidence which indicates that lysosomal enzymes might be cytotoxic in an extracellular location, in the absence of phagocytosis, seems to be lacking[17]. Furthermore, we have shown the least activity of the lysosomal marker, β-glucuronidase, in fractions richest in plasma membranes and in cytolytic activity. Our data seem consistent with a system intrinsic to plasma membranes, rather than either of these alternative mechanisms.

The cytolytic plasma membrane fractions obtained from the lymph nodes were probably in greater part from T-lymphocytes, but B-lymphocytes and other supporting tissue cells as a source have not been excluded. The lymph node cells contained fewer than 4% macrophages, and removal of histochemically detectable macrophages by adsorption on cotton wool[18] did not diminish the cytolytic capacity of isolated membranes. It remains to be examined whether the cytolytic activity is confined to the membranes of stimulated lymphocytes, the nature of the cytolytic activit of these membranes, and the role that it may have in cel mediated cytotoxicity.

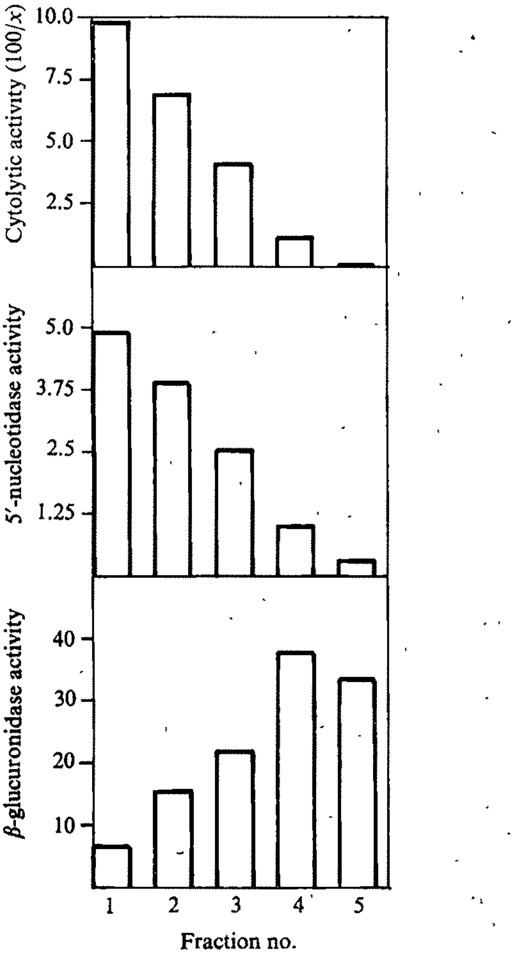


Fig. 2 Relationship between cytolytic activity, 5′-nucleotidase activity, and β-glucuronidase activity of different subcellular fractions from sensitised lymph node cells (see Table 1). One volume of packed cells (0.5–1.0 ml) was suspended in 9 volumes of saline containing 10 mM Tris-HCl buffer at *p*H 7.4 and 1 mM Mg Cl. The cells were disrupted by forcing them through a spring-loaded valve in a pump designed by Wright *et al.*[10]. One volume of the cell homogenate was mixed at 4 °C in a Teflon-rotary homogeniser with two volumes of 60% w/w sucrose in 10 mM tris at 7.4 *p*H. Five ml of this mixture was overlaid in a tube of 15×97 mm with 2 ml volumes of 39, 36, 34, 32 and 30% sucrose (w/w) solution and the tubes centrifuged in a swing-out rotor at 75,000*g* for 12 h. The fractions collected at these sucrose densities were diluted and sedimented at 70,000*g* for 1.5 h, resuspended in Tris-saline, sedimented again and finally suspended in small volumes of Tris-saline. $100/X$ is the reciprocal of the protein concentration (X, in μg) of a fraction needed for 50% lysis of mastocytoma cells (see Fig. 1). 5′-nucleotidase activity is expressed as nmoles of inorganic phosphate liberated from adenosine-5′-monophosphate (Boehringer) at *p*H 8.5 by 1 μg protein of a fraction per hour using the method of Widnell and Unkeless[12]. β-glucuronidase activity is presented as pmol of phenolphthalein released from phenolphthalein glucuronic acid per μg protein per h by the method of Talalay *et al.*[13]. The fractions are numbered as follows: $1 = < 32\%$ sucrose, $2 = 32-34\%$ sucrose, $3 = 36-39\%$ sucrose, $4 = 39-43\%$ sucrose, $5 =$ whole homogenate.

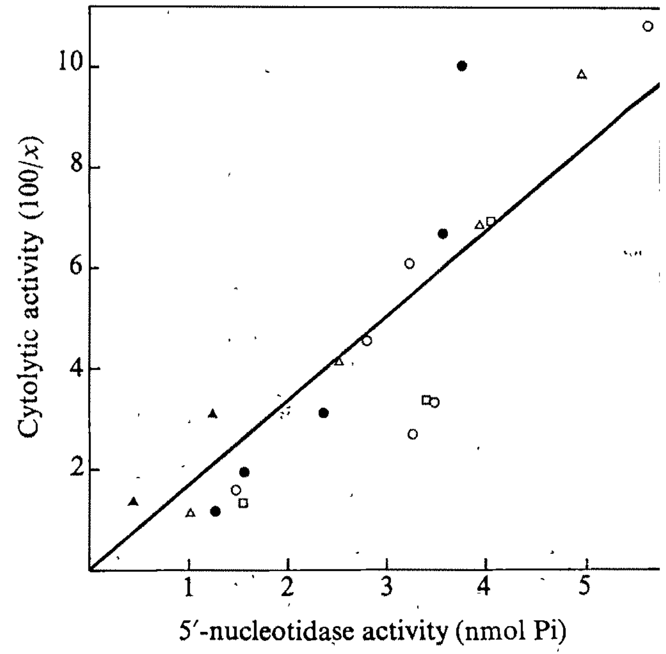


Fig. 3 The relationship of cytolytic and 5′-nucleotidase activities in several fractions from five different membrane preparations. Activities are expressed as in Fig. 2. Analysis of variance (performed by Mr M. J. R. Healey) showed that a single line through the origin provided a satisfactory fit for all the observations, with no significant differences in slope or intercept between the five sets. The slope was 0.168±0.011 showing a highly significant positive correlation ($P \ll 0.001$).

J.F. is supported by the Cancer Research Campaign.

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Received February 18; accepted May 18, 1975.

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Cytotoxicity of alveolar macrophages for virus-infected cells

MANY studies have emphasised the importance of cell mediated immunity in virus infections[1–4] and this has bee measured *in vitro* by leukocyte-mediated killing of virus infected target cells[5–8]. Although several investigators hav concluded that the active cells *in vitro* are specificall sensitised T lymphocytes[6,9], a similar activity for peritonea macrophages has been suggested[5]. The role of alveola macrophages in virus infections of the respiratory trac has, however, received little attention. Therefore, in a study of immunity to respiratory disease of cattle, the activity of cells washed from the lungs was investigated. This report describes experiments demonstrating the ability of such cells to kill parainfluenza virus-infected cells.

Lungs from freshly slaughtered calves were washed out with phosphate buffered saline (PBS) and the resulting cell suspension centrifuged at 500*g* for 20 min. The cell pellet was suspended at about 10^7 cells ml^{-1} in Eagle's Basal Medium containing 2% heated foetal calf serum (BMS). This preparation is subsequently referred to as lung wash cells (LWC). Lymph node cell (LNC) suspensions were prepared by teasing roughly minced bronchial lymph nodes in BMS and peripheral blood mononuclear cells (PBL) were isolated from heparinised whole blood by centrifugation on Ficoll–Triosil gradients[10]. The viability of all cell preparations exceeded 90%, by Trypan blue exclusion.

The cytotoxicity of these cells was estimated by measuring release of ^{51}Cr from infected calf kidney cells. The calf kidney (CK) cell suspensions were prepared by trypsinisation of primary monolayers, inoculated with at least 50 plaque forming units (PFU) of parainfluenza-3 (Pi-3) virus per cell and labelled with 40 μCi ^{51}Cr (sodium chromate, 100–300 mCi mg^{-1} Cr, Radiochemical Centre, Amersham) per 10^6 cells.

Fluorescent antibody staining of unfixed CK cells infected with Pi-3 virus showed that viral antigens were present on the cell surface in maximal amounts and in 100% of cells at 18 h. Therefore, in the cytotoxicity assay, target cells were always used at this time.

Washed, labelled CK cells (infected or uninfected) were incubated with the LWC, LNC or PBL preparations in scintillation vials for 18 h at 37 °C. After centrifugation (300*g* for 10 min) the radioactivity in cells and supernatant fluids was measured separately in a gamma counter and specific ^{51}Cr release calculated (Tables 1 and 2). Incubation of cell mixtures for longer than 18 h did not result in a significant increase in specific ^{51}Cr release.

Results of preliminary experiments are shown in Table 1. Significant specific ^{51}Cr release from Pi-3 virus-infected target cells was detected with LWC but not with LNC or PBL. Specific ^{51}Cr release from uninfected target cells never occurred. Cytotoxicity was shown by LWC from one gnotobiotic and four conventional calves which had recovered from Pi-3 virus infection. Activity was also shown by LWC from a control gnotobiotic calf, never exposed to Pi-3 virus. Further experiments (Table 2, Fig. 1) used LWC from 6 and 9-d-old normal calves and these cells showed comparable cytotoxic activity.

The spontaneous ^{51}Cr leakage from virus-infected target cells was about 30% (Table 1). By infecting and labelling target cells simultaneously as described in Table 2 leakage was reduced to about 22%, thus increasing the sensitivity of the assay. By this method specific ^{51}Cr release by LWC from calf 5 was increased from 21% (Table 1) to 38%.

Hyperimmune antiserum inhibited LWC cytotoxicity (Table 1), and this effect was examined further. At dilutions of 10^{-1} to 10^{-3} the antiserum inhibited cytotoxicity by more than 80% and inhibition was still detectable at a dilution of 10^{-4} (Table 2).

Six different ratios of LWC to target cells were investigated (Fig. 1). There was an approximate linear increase in the amount of specific ^{51}Cr release as the ratio was increased from 5:1 to 50:1. Thereafter no further increase in ^{51}Cr release was measured up to a ratio of 200:1.

These experiments demonstrate that bovine LWC have

Table 1 Cytotoxicity of various calf cell populations for Pi-3 virus-infected CK cells

Calf no.	Age (months)	Previous Pi-3 virus infection	Cell type	Antiserum*	% Specfic ^{51}Cr release† from Pi-3 virus-infected cells
Conventional 1	7.5	+	LWC	–	26.8
			LNC	–	0.4
2	7.5	+	LWC	–	26.5
			LNC	–	4.0
3	7	+	LWC	–	25.6
			LNC	–	0.8
4	8	+	LWC	–	23.9
			LNC	–	1.1
Gnotobiotic 5	2.5	+	LWC	–	20.5
			LNC	–	1.6
			PBL	–	0
			LWC	+	5.2
			LNC	+	2.1
6	1.5	–	LWC	–	16.2
			LNC	–	0
			PBL	–	0
			LWC	+	4.6
			LNC	+	0.5
Medium Controls			None	–	0
			None	+	0

Suspension cultures of 10^7 virus-infected or uninfected secondary CK cells in 25 ml BMS were incubated at 33 °C for 18 h. After washing twice at 4 °C the cells were resuspended in 4 ml BMS and incubated with 400 µCi ^{51}Cr (sodium chromate) at 37 °C for 30 min. The cells were washed four times in cold BMS, held at 4 °C for 1 h, washed again and resuspended at 10^5 cells ml^{-1} in BMS. Mixtures of 1 ml of this cell suspension and 10^7 LWC, LNC or PBL in 1 ml BMS were made in scintillation vials and the vials incubated at 37 °C for 18 h. Each vial was filled with 8 ml cold PBS and then centrifuged at 300*g* for 10 min. Each supernatant fluid (5 ml) was transferred to a clean vial for gamma counting. The remaining cells were lysed by adding 5 ml 1% sodium dodecyl sulphate in PBS and also counted. The percentage specific release of ^{51}Cr was calculated as follows:

$$\frac{\text{c.p.m. in supernatant} - \text{c.p.m. in medium control supernatant}}{\text{Total c.p.m. in cells} + \text{supernatant} - \text{c.p.m. in medium control supernatant}} \times 100$$

* Hyperimmune gnotobiotic calf Pi-3 virus antiserum, haemagglutination inhibition titre 1 : 10,000. Used at a final dilution of 1 : 100.

† Values for calves 1–4 and PBL are based on two replicates. All other values are based on four replicates. Spontaneous ^{51}Cr leakage from uninfected CK cells was 14.4–18.6% and from virus-infected cells was 29.6–32.4%.

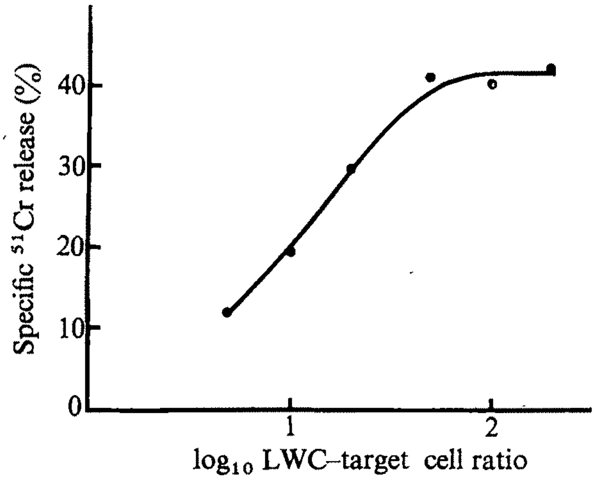

Fig. 1 Effect of different ratios of LWC to target cells on cytotoxicity. Methods are similar to those described in Table 2 except that no antiserum was used and concentration of LWC was varied from 5×10^6 to 2×10^7 cells ml^{-1}. Values are based on three replicates. Spontaneous ^{51}Cr leakage from uninfected CK cells was 16.7% and from virus-infected cells was 21.3% LWC were collected from a 9-d-old conventional calf.

a potent cytotoxic activity against virus-infected cells and this activity is inhibited by specific anti-viral serum. In addition, there is no activity against uninfected cells, and therefore exposed viral antigen in the cell membrane seems to be necessary for LWC action. The readily demonstrable cytotoxicity of LWC from two gnotobiotic calves never exposed to Pi-3 virus (16.2% specific ^{51}Cr release by calf 6, Table 1 and 17% by another control calf tested as in Table 2) shows, however, that the LWC do not require previous experience of Pi-3 virus antigens. Therefore, the LWC activity probably reflects a general reaction against 'foreign' components in the infected target cell and this hypothesis is currently under test.

Various immunological mechanisms have been described for the destruction of virus-infected cells. Complement-mediated reactions in the presence of antibody[11,12] or antibody and leukocytes[5] are unlikely to have occurred in the current work as all sera were heat-inactivated. Other antibody-dependent mechanisms involving various types of effector cell[1,7,8,13,14] are also ruled out because specific antiserum, even at high dilution, inhibits rather than enhances LWC cytotoxicity. The lysis of virus-infected cells by specifically sensitised T cells[6,9] is an unlikely explanation of our results because the LWC activity was detected in the absence of specific previous sensitisation of the donor calves, and recent experiments show that only glass-adherent cells are cytotoxic. Supernatant fluid from cocultures of LWC with target cells is non-cytotoxic, indicating that soluble factors are not involved. Therefore, it is most likely that LWC cytotoxicity is caused by macrophages[2,15–17] activated following the entry of foreign antigens into the respiratory tract. Preliminary evidence suggests that this macrophage activity increases during the first week of life paralleling the macrophage-mediated, age-dependent resistance of mice to herpes simplex virus[18,19].

Table 2 Effect of Pi-3 virus-specific antiserum on calf LWC cytotoxicity for Pi-3 virus-infected CK cells

LWC§	Final antiserum† dilution	% Specific ^{51}Cr release* from Pi-3 virus-infected cells
+	—	42.2
+	10^{-1}	6.9
+	10^{-2}	6.6‡
+	10^{-3}	7.4
+	10^{-4}	29.9‡
+	10^{-5}	41.1
—	—	0
—	10^{-1}	0
—	10^{-3}	0
—	10^{-5}	0

Secondary CK cells were suspended in either Pi-3 virus (50 PFU per cell) or medium, mixed with ^{51}Cr (40 µCi per 10^6 cells) and incubated at 37 °C for 1 h. The cells were then washed four times in cold BMS, resuspended at 10^5 cells ml^{-1} and dispensed into scintillation vials (1 ml per vial). After incubation at 37 °C for 18 h the cells in the vials were washed twice with cold BMS. One ml of BMS containing 10^7 LWC and the relevant antiserum dilution was then added. After incubating at 37 °C for 18 h the vials were treated as described in Table 1.

* Values are based on three replicates. Spontaneous ^{51}Cr leakage from uninfected CK cells was 15.9–17.8% and from virus-infected cells was 21.7–23.9%.

† Hyperimmune gnotobiotic calf antiserum as in Table 1.

‡ Mean value of spontaneous ^{51}Cr leakage from Pi-3 virus-infected control cells used to calculate these figures.

§ LWC were collected from a 6-d-old conventional calf.

Pi-3 virus is regarded as a major cause of respiratory disease in calves[20] and the destruction of Pi-3 virus-infected cells in the respiratory tract may be an important mechanism for the elimination of virus. The precise role and significance of this phenomenon in the defence of the respiratory tract against virus infection is being investigated.

We thank A. P. Collins and P. D. Luther for assistance in the preparation of calf kidney target cells. M. P. is a visiting worker from Wellcome Research Laboratories, Beckenham, Kent.

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Received April 9; accepted April 28, 1975.

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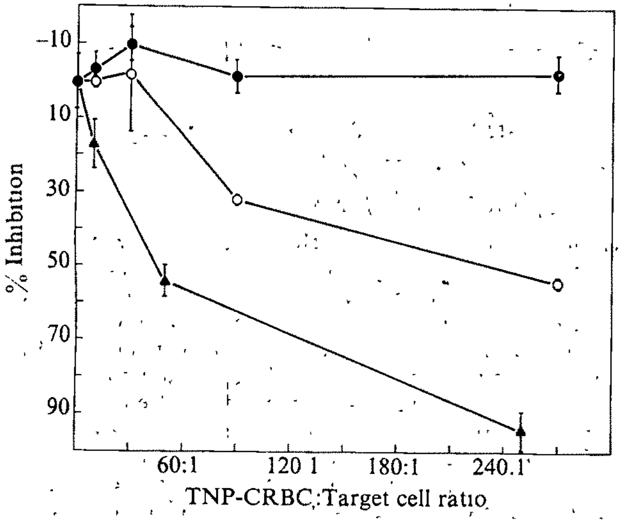
Cell-mediated immunity to hapten modified self- and non-self antigens

CELL-mediated lysis of cells infected by certain viruses or coupled with haptenic groups has been reported to occur only in situations in which the target cell and cytotoxic effector cell are either syngeneic or semi-allogeneic and cannot be observed when they are allogeneic[1–4]. Thus, spleen cells from random-bred chickens bearing Rous sarcomas are very cytotoxic to autochthonous tumour cells but show little cytotoxicity on allogeneic tumour cells. Mouse cells infected with lymphocytic choriomeningitis virus (LCM) are only lysed by sensitised thymus-derived cells (T cells) syngeneic or semi-allogeneic to the target cells[2]. Similarly, spleen cells sensitised *in vitro* to trinitrophenyl (TNP) coupled syngeneic spleen cells were reported to lyse only syngeneic but not allogeneic hapten-coupled target cells[3]. Since in all these models the induction of cell-mediated cytotoxicity was carried out in a syngeneic situation, it is clear that the phenomenon is not a result of the induction step, but is mediated through the effector step of cell-mediated cytotoxicity. It was concluded from these results that the cytotoxic effector cells recognise the modified self antigen[2–4] rather than the determinants introduced into the cell membrane (the virus or the hapten). I report here, however, results which show that TNP-specific cytotoxicity mediated by cytotoxic T cells (T killer cells) can be detected under appropriate assay conditions.

It was recently reported[5] that T killer cells able to lyse hapten-coupled target cells can be induced *in vitro* by both syngeneic and allogeneic hapten-coupled cells and that the activity of such spleen cells can be assayed on hapten-coupled targets either syngeneic or allogeneic to the immunising cells. I have chosen this model to reinvestigate the question of whether T killer cells can in fact recognise antigenic determinants artificially introduced into the cell membrane. If they can, then competitive inhibition of the cytotoxic activity should be possible using free antigenic determinants.

C57BL/6 spleen cells were sensitised (*in vitro*) to TNP-coupled syngeneic spleen cells or TNP-coupled allogeneic DBA/2 P815 mastocytoma cells (TNP-P815)[5]. On day 5 the cultures were collected and assayed for cytotoxicity on TNP-coupled C57BL/6 leukaemia EL4 (TNP-EL4) or TNP-coupled BALB/c lymphoma S49 (TNP-S49) targets[5]. Inhibition studies were performed using TNP-coupled chicken erythrocytes (TNP-CRBC) as inhibitors[5], since free hapten (TNP-lysine) does not inhibit killing of TNP-coupled targets in this model system[3,5]. This is not surprising since purified H2 histocompatibility antigens[6], in contrast to whole cells[7], do not inhibit cytotoxicity of allogeneically sensitised T cells. The cytotoxicity of C57BL/6 spleen cells sensitised to syngeneic TNP-coupled spleen cells cannot be inhibited by TNP-CRBC if the assay is done on TNP-EL4 targets (Fig. 1). Cytotoxicity of the same spleen cells, however, is inhibited by TNP-CRBC if cytotoxicity is assayed on allogeneic TNP-S49 cells. Similarly, if C57BL/6 spleen cells are sensitised to TNP-P815 and assayed on TNP-EL4, the cytotoxicity observed is again inhibitable by TNP-CRBC. This would suggest that TNP-specific cytotoxicity is only observed if the hapten-coupled cell used as target is allogeneic to the hapten-coupled sensitising cell. Two explanations can be considered: (1) within the total population of cytotoxic cells there are two distinct populations—one recognises TNP on allogeneic target cells and is inhibitable by TNP-CRBC, the other recognises modified self antigens and is not inhibitable by TNP-CRBC; (2) the population of cells which recognises modified self also recognises TNP, but the affinity of their receptors for TNP is low. Although the experiment presented cannot distinguish between these alternatives, both of them account for the observation that cell-mediated cytotoxicity to hapten or virus-modified cells is higher if the assay is done on targets which share histocompatibility antigens with the sensitising immunogen[1–5]. It seems, therefore, that a preferential sensitisation to TNP-modified self antigens occurs (refs 3 and 5, and Fig. 1). TNP-specific cytotoxicity, however, can be detected under appropriate assay conditions. This supports and extends previously published data showing that T cells can be sensitised to haptenic groups[8–11].

Fig. 1 C57BL/6 spleen cells were sensitised to TNP-coupled C57BL/6 spleen cells and TNP-P815 (ref. 5) and assayed for cytotoxicity on normal and TNP-coupled target cells at an attacker-to-target cell ratio of 100:1 (ref. 5). TNP-CRBC (ref. 5) were added as inhibitors at the different ratios shown on the abscissa. The time points were taken after 4 h (●) and 8 h (○, ▲) respectively. The percentage inhibition of the net cytotoxicity on TLP-EL4 as inhibited by TNP-CRBC is also plotted, with the s.e.m. values shown as vertical bars. Cytotoxicity was 51.3% on TNP-EL4 and 0% on EL4 for spleen cells sensitised to TNP-C57BL/6 spleen (●); 9.4% on TNP-S49; and 0% on S49 for the same spleen cells (○), 22.2% on TNP-EL4 and 5.6% on EL4 for spleen cells sensitised to TNP-P815 (▲). There was no inhibition at all by uncoupled CRBC.



This work was supported by a US Public Health Service grant from the National Cancer Institute. I thank Miss Margaret DeRose for technical help.

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Received April 14, accepted April 25, 1975.

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Comparative growth of bovine lymphosarcoma cells and lymphoid cells infected with *Theileria parva* in athymic (nude) mice

TUMOUR-LIKE growth of bovine lymphoid cells infected with *Theileria parva* has been observed in whole-body irradiated athymic (nude) mice using cells isolated direct from fatal cases of East Coast fever[1], and also using lymphoid cells infected with *T. parva*, maintained as lines in culture[2]. Here we compare the growth, in irradiated athymic mice, of this latter cell type with that of similar bovine transformed lymphoid cells of known neoplastic origin. The results contrast with those recorded in the natural conditions in cattle, since cells infected with *T. parva* grew malignantly in mice whereas bovine lymphosarcoma cells formed localised non-invasive tumours.

Twelve 6–8-week-old male athymic (nude) mice (Carworth, Huntingdon) were irradiated at 700 rad from a ^{60}Co source. Twelve similar mice were untreated. Four mice from each group were inoculated subcutaneously with bovine lymphoid (C2) cells infected with *T. parva*[3]; four from each group with bovine lymphosarcoma (BL3) cells, and four from each group with normal bovine lymphoid cells.

Tumours or tumour-like masses developed in all the irradiated

Table 1 Comparative growth of bovine lymphosarcoma (BL3) cells and (C2) cells infected with *T. parva* in athymic mice

Cell–host reaction	BL3 cells	C2 cells
Growth in athymic mice		
Non-irradiated	None	Occasional limited growth
Irradiated	Very good. No regression observed	Very good. Occasional regression with recovery
Growth first detectable	Days 4–7	Days 2–3
Nature of growth	No infiltration of cells, growth localised at site of inoculation. No ulceration only slight necrosis of tumour	Extensive infiltration and destruction of local musculature. Ulceration and necrosis of tumour common, sometimes extensive
Cellular dissemination	None detected on histology or organ impression smears	Parasitised cells commonly seen in blood, lung and liver; also in kidney, spleen, brain and lymph nodes
Clinical symptoms in host	No generalised symptoms	Debility, wasting, terminal respiratory distress and anorexia
Host death	Not recorded	Common 30–50 d
Passage of tumour cells to athymic mice		
Non-irradiated	None	Occasional limited growth
Irradiated	About 80% take	About 80% take

The details recorded represent the collected observations from 11 mice (BL3 cells) and 50 mice (C2) cells. BL3 cells were obtained from fatal case of lymphosarcoma[8] in an adolescent steer (Code 8004) and were supplied by the Cell Culture Laboratory, Naval Biomedical Researc Laboratory, Oakland, California at passage 1. A pure lymphoblastoid cell suspension was isolated in this laboratory, and incoculated int mice at passage 10. C2 cells were passage 24. Both cell lines were grown as described previously[3] and each mouse received 5×10^7 cells in 0.2 m medium from day 2 static cultures. Control mice received similar inocula of normal bovine lymphoid cells isolated by the method of Thorsb and Bratlie[9] from 100 ml of normal bovine blood collected by venipuncture into 50 IU heparin ml^{-1}. Inoculations were given approximatel 1 h after collection of blood.

mice inoculated with C2 or BL3 cells, but not in any other groups. The growth of C2 cells and the responses of the host were similar to those described previously[1,2]. In the irradiated mice inoculated with BL3 cells, tumours were first detected at the site of inoculation on day 7; they continued to grow as localised masses and became very large. There was no evidence of vascular spread of cells and mice remained clinically normal until the tumours became so large that they hampered movement and inhibited feeding and drinking. This stage was reached between days 50 and 70, and mice were then destroyed on humane grounds. BL3 cells were passaged directly to further athymic mice as described previously[1], and a similar growth pattern was recorded: tumours occurring in irradiated mice, but not in untreated mice.

Table 1 summarises the different growth patterns of C2 and BL3 cells in athymic mice, recorded in the above and other experiments. BL3 cells formed discrete circumscribed tumours with no evidence of metastasis, whereas C2 cells infiltrated extensively causing a malignant neoplastic condition. These findings contrast with the natural conditions in cattle—lymphosarcoma being a malignant neoplasia—and East Coast fever, a non-malignant condition with no evidence of tumour formation.

C2 and BL3 cells grew consistently only in irradiated athymic mice, suggesting that non-irradiated mice retained some cell-mediated immune activity which was abolished by irradiation. Residual T cell activity in athymic mice has been demonstrated[4-6], and the present work further indicates the functional nature of these cells in non-irradiated athymic mice. Because of its nonspecific action, however, irradiation also suppresses B cell activity and thus antibody production. This may be significant in the present context since anti-tumour antibodies have recently been demonstrated in the sera of athymic mice[7]. Irradiation would presumably inhibit production of such antibodies; anti-tumour activity could then be abolished if sufficient tumour antigens were inoculated to eliminate circulating antibodies.

We thank Dr J. Weaver for supplying BL3 cells and Professor G. Mugera for allowing us irradiation facilities. The work was supported by the International Atomic Energy Agency. The project is supported by the UN Development Programme with the FAO as the Executin Agency in cooperation with the East African Community.

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Nonspecific cross reacting antigen as a marker for human polymorphs, macrophages and monocytes

WE have described[1] an antigen able to cross react with th carcinoembryonic antigen of the digestive system (CEA and named it nonspecific cross reacting antigen (NCA because of its reaction with CEA and its lack of tissue an cancer specificity. NCA was found in perchloric extract of gastrointestinal tumours and in lesser amounts in thos of non-cancerous mucosa, lung and spleen. The sam antigen has been described under different names (NG CCEA II) in other laboratories[2,3] We pointed out that NC bore a specific antigenic determinant not shared by CEA[1].

This has provided us with the possibility of raising specific rabbit anti-NCA antiserum, and of using it to study the localisation of NCA by indirect immunofluorescence the second layer being a fluoresceinated sheep anti-rabbit globulin antiserum (Institut Pasteur, Paris). We have already shown the presence of NCA at the apical pole of glandular cells of gastrointestinal tumours and non-cancerous colonic mucosa[4]. When studying other organs, we found that NCA is a normal component of polymorphs and of mononuclear cells looking like macrophages and monocytes. Similar observations have been made independently[10].

When examining frozen sections of human lung, we observed that many cells scattered in the alveolar walls were positively stained with anti-NCA antiserum. We therefore studied fixed cultured alveolar macrophages and found that they reacted strongly with the same antiserum, showing a cytoplasmic fluorescence, either diffuse or in the form of coarse granules.

On spleen frozen sections many cells were positive with anti-NCA antiserum, almost all of them being outside the lymphoid follicles. Spleen imprints enabled a better characterisation of the fluorescent cells: most of them were polymorphs, some were large mononuclear cells (Fig. 1).

While blood cell smears (the red cells had been lysed by hypotonic ammonium chloride) showed many positive cells. As on the spleen imprints, almost all the polymorphs were fluorescent, together with some large mononuclear cells. Small lymphocytes were always negative. The specificity of the reaction was assessed using NCA-absorbed antiserum, staining only eosinophils, which nonspecifically bind any fluorescent globulin.

The positive mononuclear cells were more numerous on smears made with the lymphocyte layer obtained after centrifugation of the blood on Ficoll–Triosil which enabled more careful study. Most of these cells were large, the same size or larger than polymorphs, with large nuclei, sometimes eccentric, and often slightly incurvated. Their fairly abundant cytoplasm gave a fluorescence which varied in intensity, and was generally diffuse or finely granular. Its tinctorial properties could not be determined precisely, as the Giemsa reagent did not stain the cytoplasm after fluorescent labelling.

To identify these NCA-positive mononuclear cells, we used different ways: Adherence to glass wool columns was uneven, some cells being able to bind the column and others not. In contrast, all cells fixed to nylon wool columns. They were not able to phagocytose iron carbonyl particles, nor aggregated γ-globulins conjugated to fluorescein (in this latter case, after the phagocytosis step, the cells were fixed with ethanol, then labelled with rabbit anti-NCA antiserum and Rhodamin-conjugated anti-rabbit globulin. Many polymorphs were observed containing green granules in a diffusely red cytoplasm; mononuclear cells were only red). EA rosettes were prepared, recovered by centrifugation on Ficoll–Triosil, and smeared. Among the many mononuclear cells, a part was stained by anti-NCA antiserum. The positive cells were much more numerous than on ordinary Ficoll–Triosil smears, indicating that NCA-positive cells were engaged in EA rosetting and concentrated by this procedure, and therefore had Fc receptors on their surface. We looked for intracytoplasmic immunoglobulins using double fluorescence on Ficoll–Triosil and EA rosette smears, and concluded that many, if not all, NCA-positive mononuclear cells did not contain detectable immunoglobulins and that Ig-positive cells were negative for NCA.

It is thus likely on morphological and physiological grounds that NCA-positive mononuclear cells belong to the monocyte series, and that they could be considered as ancestors of the macrophages found in lung alveolar walls. They do not, however, have all the characteristics classically attributed to monocytes as they lack phagocytic activity. Yet it is quite possible that young monocytes also do not have all these properties. Note that mouse K cells have been described as young monocytes[5], without phagocytic activity. Yet human K cells differ from NCA-positive cells as they do not adhere to nylon columns[6]. Definitive identification of NCA-positive cells could be obtained by electron microscopy, using, for example, peroxidase-labelled antibodies.

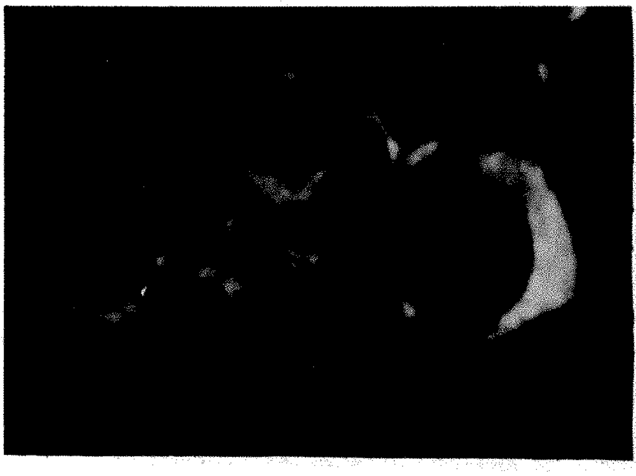

Fig. 1 Spleen imprint: three cells, two polymorphs and a large mononuclear cell, stained with anti-NCA antiserum.

NCA-containing cells were also observed in bone marrow smears, and belonged mainly to the myeloid series; leukaemic myeloblasts were also positive. In ascitic and pleural fluids, polymorphs and large mononuclear cells were also fluorescent.

NCA has thus been found in the cytoplasm of polymorphs and large mononuclear cells. Its subcellular localisation is still to be determined, but it is tempting to speculate that this antigen could belong to the lysosomal system. If so, NCA could equally well be a component of the lysosomal wall as an intralysosomal enzyme. The high carbohydrate content of the NCA molecule makes the first hypothesis more likely. Here again, electron microscopic studies using peroxidase-labelled antibodies could be helpful in determining precisely the subcellular localisation of NCA.

NCA was also found on the surface of polymorphs and large mononuclear cells. This was shown in two ways: membrane fluorescence on living cells, which gave brighter images on polymorphs than on mononuclear cells, and agglutination of the white cells by anti-NCA antiserum. When diluted antiserum was used, small agglutinates were obtained, which could be smeared and stained by Giemsa reagent: they contained mainly polymorphs, and large mononuclear cells looking like monocytes. NCA-absorbed antiserum did not agglutinate the cells, nor stain their surface. Thus the specificity of the reaction could be assessed.

As mentioned above, there is an antigen common to polymorphs and mononuclear cells, which are probably monocytes, that is absent from other white blood cells. This result fits well with the experiments of Metcalf[7], and Morley *et al.*[8] showing that there is in the bone marrow an ancestor cell common to polymorphs on one side, and macrophages and monocytes on the other side, the remaining white cells being derived from different stem cell lines.

In conclusion, we have shown that NCA is present in the cytoplasm and on the surface of human polymorphs and monocytes, and in the cytoplasm of macrophages. We believe that this is the first time that a surface marker has been described for human polymorphs. The role of this new marker in characterising normal and pathological human white cells will be studied further. It can for instance be compared with the chloroacid esterase method[9] known to be very useful in the diagnosis of myeloblastic leukaemias.

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Breakage of tolerance to α foetoprotein in monkeys

α FOETOPROTEIN (AFP) is a foetal protein produced in large quantities by the foetal liver, yolk sac, and certain tumours such as hepatomas and teratomas[1,2]. AFP does not cause immune response in a tumour-bearing host or in situations in which a transient increase in AFP takes place[2]. This apparent tolerance, however, can be broken in rabbits or mice by immunisation with heterologous[2–4] or chemically modified homologous AFP (ref. 5). Both these approaches have been found effective in breaking tolerance to other antigens[6]. It has been shown that AFP-producing hepatoma cells are killed by anti-AFP antibodies *in vitro*[7,8]. AFP may thus be of potential use as target antigen in tumour immunotherapy.

We show here that tolerance to autologous AFP can be broken in monkeys by immunisation with rabbit AFP. It is likely that the same procedure could be used in humans should animal experiments warrant such an approach to immunotherapy of AFP-producing human tumours.

Our choice of rabbit AFP for immunisation of monkeys was based on the observation that human (and monkey) AFP shows strong immunological cross reactivity with rabbit AFP when sheep antisera are used. Two monkeys, one *Cercopitheus ethiops* (Vervet) and one *Macaca irus* (Cynomolgus), were immunised with purified rabbit AFP (ref. 9). Three injections of 0.5 mg AFP in Freund's complete adjuvant were given 2 weeks apart and the animals were bled 2 weeks after the last injection. Antibody response against AFP was measured by immunodiffusion and/or radioimmunoassay[10]. In the latter test we used human or rabbit AFP as the labelled antigen. This is justified in view of the striking immunological similarity of human AFP and AFP from the monkeys used (Fig. 1). Concentration of AFP from monkey sera was achieved by adsorption to and elution from sheep anti-human AFP immunoadsorbents[9].

Both monkeys produced strong precipitating antibodies against rabbit AFP (Fig. 2) and the antisera also reacted with purified human AFP and the *Cercopitheus* and *Macaca* sera containing AFP, but not in a detectable manner with normal sera from the same species. High binding titres were obtained with rabbit and human AFP in radioimmunoassay with the majority of antibodies directed against the immunogen (Table 1). As seen in Fig. 2, the monkey anti-AFP sera react with lines of identity against homologous monkey AFPs as well as against human AFP.

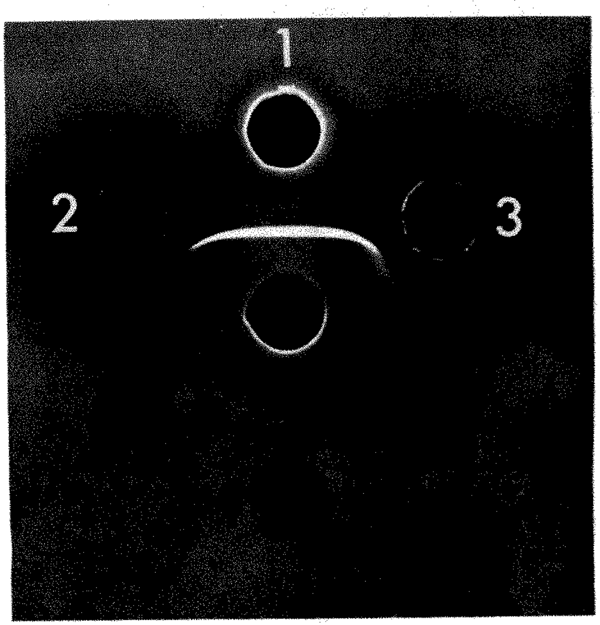

Fig. 1 Immunodiffusion in agarose. 1, Human foetal serum; 2, AFP-positive serum from a hepatoma-bearing *Cercopitheus* monkey; 3, a similar serum from a *Macaca* monkey; centre well, sheep anti-human AFP.

Further evidence that the antibodies produced by the monkeys immunised with rabbit AFP also reacted with their own AFP, was provided by experiments showing that these antisera lacked free AFP. Using immunoadsorbents it is possible to isolate small amounts of AFP from normal monkey sera, whereas no detectable AFP was recovered from the immune sera (Table 2).

We have thus succeeded in producing antibodies to autologous AFP in monkeys. This is in accordance with similar data obtained in rabbits[5] and mice (E. Ruoslahti and H. Pihko, unpublished). It has recently become evident that many other oncofoetal antigens by themselves may cause an immune response in multiparous animals or tumour bearers. Such antigens characteristically behave as group specific antigens, that is, a common antigen is shared by tumours arising from a given cell type[12–14]. Human tumour antigens demonstrable by cytotoxicity of autologous lymphocytes show similar tissue specificity[15] and it has been suggested that they represent foetal antigens. Possible immunogenicity of foetal antigens may thus be of considerable importance in tumour immune reactions.

Why AFP is not immunogenic by itself in its host of origin may be explained by the extremely high foetal levels

Fig. 2 Immunodiffusion in agarose. Left: anti-AFP from *Cercopitheus*; right: anti-AFP from *Macaca*. 1, Purified rabbit AFP; 2, purified human AFP; 3, AFP-positive *Cercopitheus serum*; 4, AFP-positive *Macaca* serum; 5, normal *Cercopitheus* serum; 6, normal *Macaca* serum.

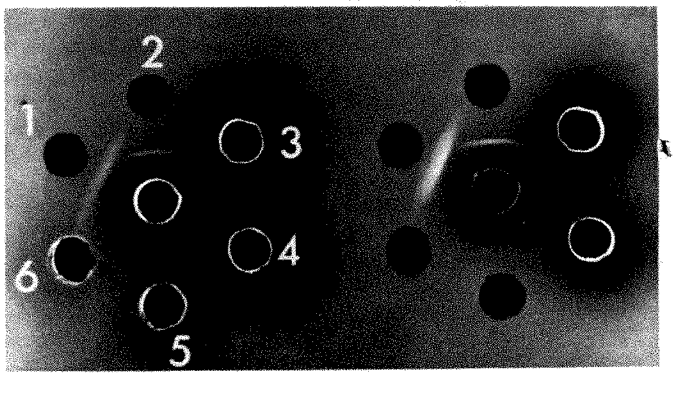

Table 1 Binding of ^{125}I-labelled rabbit and human AFP by monkey anti-rabbit AFP sera

Serum	Titre* with rabbit AFP	Titre* with human AFP
Cercopitheus anti-rabbit AFP	9×10^4	9×10^2
Macaca anti-rabbit AFP	3.5×10^4	3×10^2

* Reciprocal of serum dilution binding 50% of the label precipitable at antibody excess.

(up to several mg ml^{-1}) and the residual small concentrations in adult serum (a few ng ml^{-1} in humans[10,11], about 100 ng ml^{-1} in mice[16] and rabbits[5]).

It is likely that these AFP concentrations are much higher than is the case for those oncofoetal antigens which cause an immune response in adult animals. These latter antigens may also only be expressed during an earlier embryonic period than AFP, thus allowing more complete loss of immune tolerance[17].

AFP is, unlike most other oncofoetal antigens, a well characterised molecule. Several animal models are available for studies, thus enabling accurate measurement of various aspects of AFP expression and manipulation of its immunogenicity. Recent results from several laboratories show that in spite of the fact that AFP is secreted by hepatoma cells, such cells can be killed using heterologous anti-AFP (refs 7 and 8). The limited attempts to show tumour resistance in animals treated with anti-AFP or immunised against their own AFP have given inconclusive results[4,8], but definitive degenerative changes have been demonstrated in the liver of foetuses from rats immunised against their own AFP (ref. 18). These results suggest that further studies on breakage of tolerance to AFP and the effect of the induced antibodies on the appearance and growth of AFP-producing tumours may prove rewarding.

Table 2 Radioimmunoassay of AFP isolated from normal and anti-rabbit AFP monkey sera using immunoadsorbents*

Sample		Amount of AFP (ng): Original sample	Eluate
Cercopitheus	Normal	25	10.0
	Anti-rabbit AFP	—†	< 1.0
Macaca	Normal	74	10.3
	Anti-rabbit AFP	—†	< 1.0

* A 25 ml sample of monkey serum was treated with 18% sodium sulphate to remove gammaglobulin and the supernatant was incubated for 3 d with 50 µl Sepharose conjugated anti-human AFP. The particles were eluted with 8M urea and analysed for AFP in radioimmunoassay[5,8].

† The original AFP content of anti-rabbit AFP sera could not be measured because of interference in the assay by the residual anti-AFP antibody present in the sodium sulphate supernatant.

We thank Dr D. Dalgard, Hazleton Laboratories, Vienna, Virginia, for monkey hepatoma sera, Mr Y. Kawanowa for assistance and the Finnish Cancer Foundation, the Cilag-Chemie Foundation, the Finnish MRC and the Swedish Cancer Society for support.

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Localisation of caps on mouse B lymphocytes by scanning electron microscopy

WE have used cathodoluminescence (CL) to detect fluorescein-labelled anti-IgG-induced caps on mouse B lymphocytes by means of the scanning electron microscope (SEM). This approach, which has as yet been little explored, depends critically on efficient collection of a weak CL signal[1,2]. It

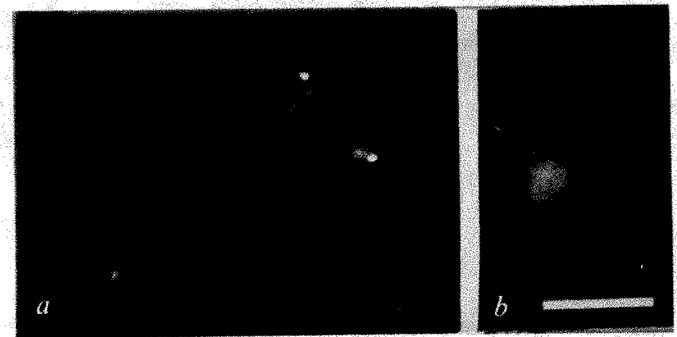

Fig. 1 Fluorescence micrograph of capped mouse lymphocytes obtained after incubation with fluorescein-labelled goat anti-mouse IgG. Bar = 10 µm. *a*, Unfixed; *b*, after fixation in glutaraldehyde, dehydration and critical point drying. Unfixed cells from lymph nodes of C3H mice were incubated with fluorescein-labelled goat anti-mouse IgG (Hyland) at 37 °C for 20 min. Some of the cells were then examined in an ultraviolet microscope to determine if they had acquired caps. The cells were then fixed in 2.5% buffered glutaradehyde for 30 min, washed and dehydrated for 10 min in each of the following: 30%, 50%, 70%, 95% and 100% ethyl alcohol. Indexed copper grids (mesh 200), coated with Formvar and a light layer of carbon were then placed on top of a Millipore filter (FLG PO 1300, porosity 0.25 µm). The cell suspension in 100% alcohol was then passed through the filter so that some of the lymphocytes settled on the grids and others on the filter. Since Formvar and carbon coated grids showed no fluorescence, the cells on the grids could then be examined for CL as well as in the secondary electron mode. Cells on the filter could only be examined by the secondary electron mode because of the autofluorescence of the filter. A holder containing the Millipore filter and grids, with adherent lymphocytes, wet in absolute alcohol, was transferred to a critical point dryer (SAMDRI TM PVT-3). Liquid CO$_2$ was then introduced slowly into the critical point dryer chamber, and left flowing through the chamber for 10–15 min to remove alcohol. The temperature of the chamber was then raised and when it reached 40 °C, the pressure was decreased at a rate of 100 pounds min^{-1}. The grids were removed and mounted on special stubs with Aquadag, for examination in the SEM (Stereoscan Mark II fitted with a sensitive CL detector system[1]). The special stubs had been drilled with holes of diameter 2 mm, painted inside with Aquadag, and the grids were mounted over those holes, to reduce CL emission from the stub material underlying the specimen. The SEM was operated at 20 kV with a beam current of 150 µA. After the uncoated lymphocytes had been examined in the secondary electron mode and for CL, the same cells were then coated with gold and examined again in the secondary electron mode. Since gold coating tends to absorb emitted light, the best CL micrographs were obtained on the uncoated samples. The images obtained by CL could be compared with secondary electron micrographs taken on the same cells before and after coating with gold. Polaroid film (type 42) was used at exposure times of 40 s for all three modes.

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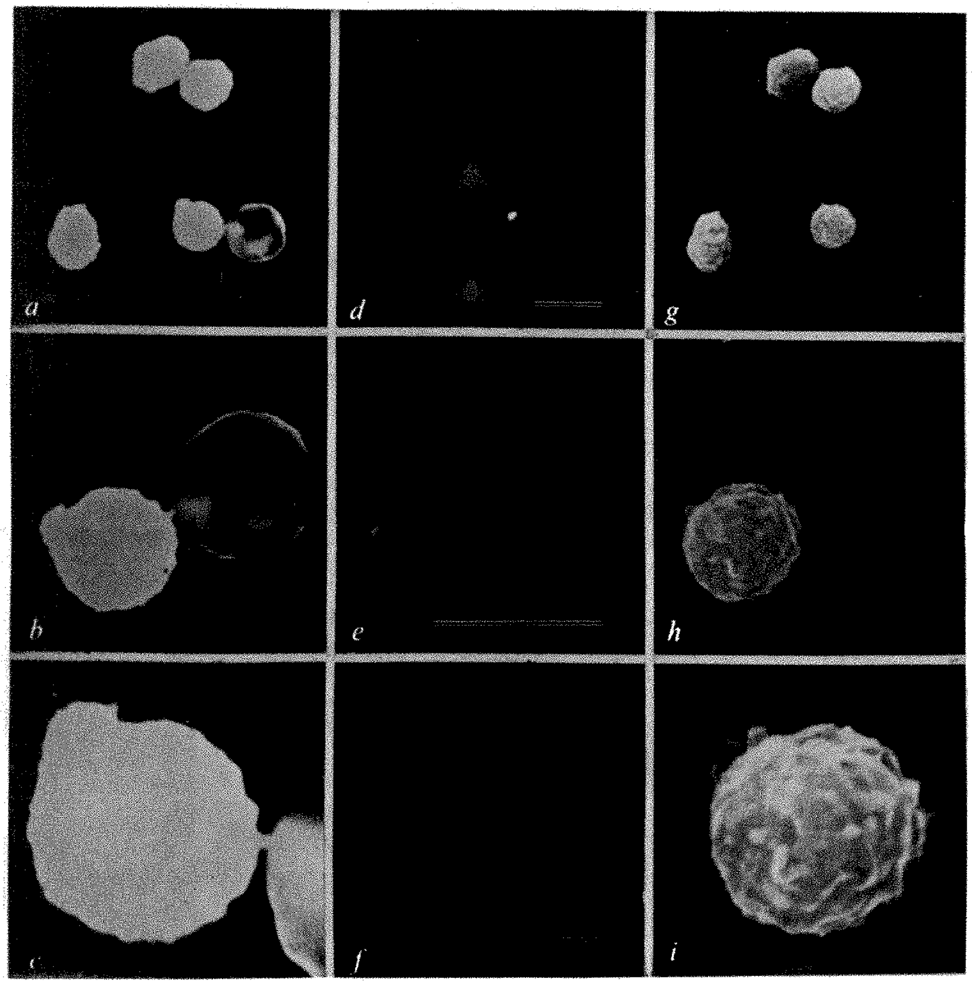

Fig. 2 Electron micrographs of the same capped lymphocyte at different magnifications. *a–c*, Uncoated, secondary electron image; *d–f*, uncoated, cathodoluminescence; *g–i*, gold coated, secondary electron image. Bar = 5 μm for *d* and *e*, 1 μm for *f*. *a* and *g* at same magnification as *d*, *b* and *h* as *e*, *c* and *i* as *f*.

provides a means of identifying antigenic markers in the SEM.

Ultraviolet microscopy showed that 20% of unfixed cells had acquired caps in our incubation conditions (Fig. 1*a*). The caps remained visible after fixation in glutaraldehyde and critical point drying (Fig. 1*b*). The same preparation examined by SEM using CL showed a similar proportion of capped cells. In CL micrographs (Fig. 2*d–f*), the cap appeared as a bright semicircular fluorescent region. At lower magnifications (up to ×2,000) the caps appeared brighter and faded more slowly than at the higher magnifications (×5,000 or ×10,000).

In secondary electron images (Fig. 2*a–c*) the surface features of the uncoated cells were not clear. When the same cells were coated and then examined, however, ridges and microvilli could be seen on their surface (Fig. 2*g–i*). The cap region of the lymphocytes, when examined in greater detail at higher magnifications, appeared as a protrusion, or bulge, on one side of the cell (Fig. 2*i*). This view of the cap agrees with observations made from electron micrographs of sections through the capped region located by ferritin and ^{125}I-labelled anti-Ig antibodies[3,4]. The cell surface in the cap region also showed several microvilli.

Thus CL can localise caps on mouse B lymphocytes under the SEM. Without the accompanying CL image it was not possible to distinguish cap areas with certainty. This technique enables immunological investigation to be made in the SEM similar to that carried out by ultraviolet fluorescence microscopy. Moreover, the same reagents—fluorescein-labelled antibodies—can be used in both techniques.

Revel *et al.* have shown that haemocyanin can be recognised in the SEM and perhaps ferritin also[5,6]. These markers and latex particles[7] can, therefore, be useful for labelling antibodies to localise antigens by means of the SEM. Our study suggests that fluorescein-labelled antibody may also prove a useful immunological marker for demonstrating surface antigens in cell preparation with the SEM using CL. Our procedure can, in principle, extend the limits of resolution beyond those obtainable with the ultraviolet microscope. Fading of the CL image at magnifications of ×10,000 or above seems to be the chief factor limiting CL resolution. The resolution we obtained in the CL mode was comparable with that obtained by ultraviolet microscopy. If antibody could be labelled so as to give more intense or stable CL than is possible with fluorescein isothiocyanate, resolution in the CL image may be improved.

We thank E. Bond for technical assistance and for designing the specimen stub and L. Subrahmanyan for preparing cells. This work was supported by the National Research Council and the Medical Research Council of Canada.

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Immunogenicity of mouse trophoblast and embryonic sac

ALTHOUGH it is generally accepted that the survival of the mammalian embryo as an intrauterine allograft can in part be attributed to the antigenic neutrality of the trophoblastic barrier of the placenta, the way in which this is achieved is unknown. It has been proposed that the trophoblast may either have an intrinsic deficit of transplantation antigens[1] or that these are present but masked in some way, possibly by a mucosubstance on the cell surface[2]. Apparently convincing evidence for this latter hypothesis came from experiments by Currie *et al.*[3] who reported that incubation with the enzyme neuraminidase rendered mouse trophoblast immunogenic on injection into recipient mice. As neuraminidase cleaves terminal 'sialic' (*n*-acetyl neuraminic) acid from underlying amino sugars[4], it was suggested that antigens were masked by sialomucin on the trophoblast surface. These findings have, however, been disputed[5] and the issue is unresolved. We now report the results of a collaborative study, using two different experimental approaches, which demonstrates that mouse trophoblast, at least at the early proliferative stage, does not possess any detectable quantity of immunogen masked by a neuraminidase-sensitive cell coat. In contrast, embryonic tissue from the same conceptus is highly immunogenic in similar test situations.

In the first series, groups of C57BL (H-2^b) mice were challenged with A(H-2^a) strain embryonic tissues and their immunogenicity assessed using a modification of a cell-mediated microcytotoxicity assay[6]. At 7.5 d *post coitum* A strain ectoplacental cone (EPC) trophoblast was carefully dissected from its embryonic sac and incubated with *Vibrio cholerae* neuraminidase (Behringwerke) at 250 U ml^{-1} for 1 h at 37 °C. C57BL mice received five successive grafts of five to eight treated EPC to sites beneath the capsule of kidneys and testes. Each C57BL recipient received a total of not less than 28 EPC. Control C57BL mice were challenged with similar serial transfers of 7.5 d. A strain untreated EPC or embryonic sacs from the same conceptus. Twelve days after the last transfer, the mice were killed, their testes and kidneys examined for evidence of graft proliferation and their spleens made into a cell suspension for testing in the cell-mediated microcytotoxicity assay at spleen cell–target cell ratios of 500 : 1 and 100 : 1.

Spleen cells from mice challenged by serial transfer of both untreated EPC and neuraminidase-treated EPC were incapable of killing target embryonic fibroblasts, whereas those from mice challenged with embryonic sac showed a significant degree of killing at identical spleen cell levels (Table 1). A differential immunogenicity of the two components of the mouse conceptus is therefore detectable using the *in vitro* cell-mediated cytotoxicity assay, and pretreatment of EPC with neuraminidase does not expose any masked antigens. Histological examination of the final site of EPC transfer revealed the presence of trophoblastic giant cells in a haemorrhagic nodule even when neuraminidase incubation had been used. The dosage of enzyme used (250 U ml^{-1}) is thus not detrimental to trophoblast viability.

Table 1 Effect of spleen cells from C57BL mice challenged with A strain embryonic sac or EPC tissue on A strain fibroblasts *in vitro*

Immunisation	Cytotoxic effect*
None	—
Ectopic EPC	—
Ectopic neuraminidase EPC	—
Ectopic sac	+++

*Cytotoxic effect scored as +++ total destruction, ++ extensive, + slight, as compared with control cultures, scored —. Scoring based on the assessment of 12 replicate wells in each of three separate experiments. Fibroblasts seeded at 10^3 or 10^4 cells per well in Cooke microtitre plates in medium RPMI +10% FCS, and allowed to plate out for 24 h before the addition of 5×10^5, or 10^6, spleen cells, respectively. Cultures were fixed, stained and assessed after a further 48 h.

Table 2 Effect of neuraminidase on the immunogenicity of mouse EPC and embryonic sac: survival of (C3H×C57BR) F_1 tail skin grafts on pre-challenged mice

C3H recipient treatment	No. of recipients	Mean skin graft rejection time (d)±s.d.
None	6	*13.7±1.21
(C3H×C57Br) F_1 skin graft	8	7.3±0.5
C3H×C3H embryonic sac	8	12.6±1.5
C3H×C3H EPC	5	13.0±1.9
(C3H×C57BR) F_1 embryonic sac	7	* 8.7±1.7
(C3H×C57BR) F_1 EPC	8	12.7±1.3

*$P = 0.001$.

In a second series, C3H/He-mg strain male mice were challenged with the F_1 embryonic tissues from C3H/He-mg female×C57BR/cd male matings, and the immunogenicity of the tissues was then assessed by a test skin graft. These strains differ at multiple strong non-H-2 loci, but are both H-2^k. (C3H×C57BR) F_1 EPC or embryonic sacs were incubated separately with *Vibrio cholerae* neuraminidase (Behringwerke 100 U ml^{-1}) for 0.75–1 h at 37 °C, washed extensively and injected intraperitoneally into C3H strain recipients. Each recipient received six embryonic sacs or EPC in phosphate-buffered saline (PBS), a repetition of the schedule used previously[3,5]. Cell viability after incubation was 75–80% on quadruplicate samplings. Control groups of C3H mice were challenged either by (C3H×C57BR) F_1 skin grafting, by intraperitoneal injections of either C3H neuraminidase-treated EPC or C3H neuraminidase-treated embryonic sacs, or had no treatment. After 7–8 d the C3H mice received a (C3H×C57BR) F_1 tail skin graft, and the rate of skin graft rejection was assessed (Table 2). Challenge with neuraminidase-treated (C3H×C57BR) F_1 cones resulted in a skin grafting rejection time of 12.7 d, which is not significantly different from that of control mice challenged with syngeneic embryonic tissues nor from the response to a first set (C3H×C57BR) F_1 skin graft, whereas challenge with neuraminidase-treated (C3H×C57BR) F_1 embryonic sacs accelerated skin graft rejection to a level comparable with that of a second-set reaction (P=0.001).

Neuraminidase-treated EPC, as well as untreated trophoblast, thus failed to immunise effectively a recipient when tested either by skin grafting or by cell-mediated lysis of target cells *in vitro*. In contrast, the other component of the post-implantation conceptus, the embryonic sac, was clearly immunogenic in these conditions. This implies that the sac possesses those determinants involved in the stimulation of lymphocyte proliferation as well as transplantation alloantigens, whereas trophoblast must be deficient in at least one of these. Other recent studies have shown that there are no detectable transplantation antigens on mouse EPC trophoblast, as demonstrated by its insusceptibility in an *in vitro* cell-mediated cytotoxicity test[7], which requires only alloantigenic differences.

The failure to unmask transplantation antigens on neuraminidase-treated EPC is in accord with the report from Simmons' laboratory[5], and it is difficult to reconcile these findings with the earlier claim of Currie *et al.* It is highly improbable that masking of trophoblast transplantation antigens is a strain-specific phenomenon and higher levels of neuraminidase than those used in the present investigations are detrimental to trophoblast cell viability[5,7]. The effectiveness of the enzyme treatment in removing cell-surface sialomucin was also confirmed by electron histochemical methods carried out in parallel on EPC outgrowths *in vitro*. The degree of colloidal iron binding to trophoblast cell-surface mucoprotein was significantly reduced after neuraminidase incubation. A number of possible explanations have been advanced to account for the differing reports[7,8], including contamination of the EPC inoculum by embryonic sac material, which has been demonstrated to be rendered even more immunogenic after enzyme treatment[5]. There is also the possibility of contamination by maternal cells, which was avoided in the present study by the use of F_1 trophoblast.

The finding of a differential immunogenic status of mouse EPC and embryonic sac may be related to their early differentiation[8]. Only weak transplantation antigens, the non-H-2 histocompatibility antigens, have so far been detected on the preimplantation mouse blastocyst[9], and it has been suggested that the antigenic component may in fact be the inner cell mass or its derivatives rather than the outer trophectoderm layer, which gives rise to trophoblast[10,11]. This is supported by the finding that antigens are not detectable on trophoblast cells after attachment and outgrowth of the blastocyst *in vitro* either by a cell-mediated cytotoxicity test[7], indirect immunofluorescence (ref. 12 and M.H.J., unpublished) or peroxidase-labelled alloantisera binding (R.F.S., and E.J.J., unpublished).

It can be concluded that early proliferating EPC trophoblast does not possess detectable transplantation antigens. This may not necessarily be the case for differentiated trophoblast of the mature placenta, although the presence of such antigens has not been unequivocably demonstrated as yet. The possible masking effect of overlying membrane-bound mucoproteins at this later stage must also be considered[13].

We thank Dr W. D. Billington for advice and Miss V. A. Merry for assistance. Financial support was provided by The Rockefeller Foundation and a project grant (to M.H.J.) from the MRC.

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Specific roles for platelet surface glycoproteins in platelet function

THE haemostatic role of the blood platelet is largely dependent on its ability to adhere to exposed subendothelial components in the event of vessel damage[1] and respond to specific aggregation-inducing stimuli, such as adenosine diphosphate (ADP), which seem to act at defined receptor sites on the platelet membrane[2]. The early stages of platelet adhesion and aggregation are surface-mediated phenomena and much interest has centred on determining which of the surface groupings are involved in these mechanisms. Iodination techniques[3,4] have revealed that a limited number of proteins are exposed on the surface of the platelet and that among these are the major membrane glycoproteins. A possible involvement of carbohydrate groupings in the mechanisms of aggregation as induced by ADP and 5-hydroxytryptamine was described by Mester *et al.*[5] who showed that the velocity of aggregation was altered by changes in the sialic acid content of the platelet.

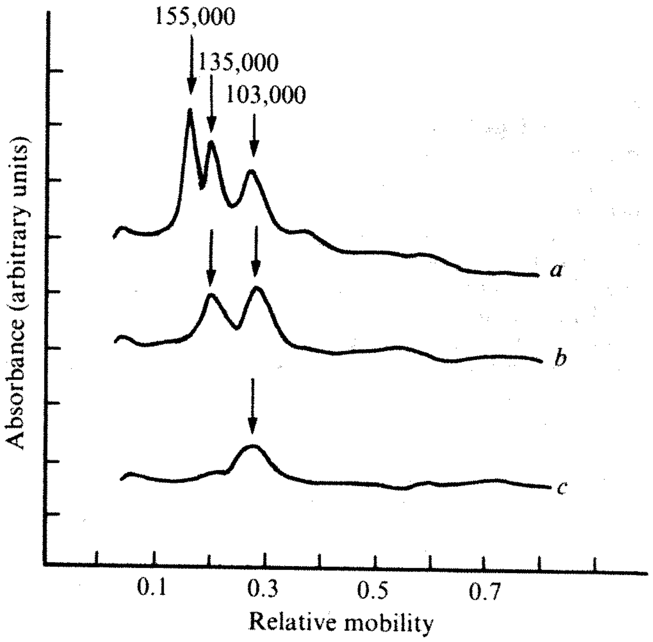

Fig. 1 Polyacrylamide gel electrophoresis in the presence of SDS showing the platelet membrane glycoprotein distribution after the digestion of washed human platelets with trypsin for 0 min (*a*); 3 min (*b*); and 15 min (*c*) before lysis and membrane fractionation. Washed human platelets suspended at 2×10^9 ml^{-1} in 0.01 M Tris HCl, *p*H 7.4, containing 0.15 M NaCl and 3 mM EDTA were incubated at 37 °C for 2 min followed by the addition of trypsin (0.2 mg ml^{-1}, Type I Sigma). At selected time intervals aliquots were withdrawn and added to soybean trypsin inhibitor (0.4 mg ml^{-1}, Type I-S Sigma). The platelets were sedimented at 2,000*g* at 4 °C and the supernatant removed. The trypsin-treated platelets were resuspended at 2×10^9 ml^{-1} in the above buffer and platelet lysis by freeze–thaw and sonication was performed as described previously[7]. Intact platelets and large particles were removed by centrifugation at 2,000*g* for 15 min at 4 °C. The supernatant in 2 ml aliquots was centrifuged at 65,000*g* for 1 h at 4 °C over a 0.5 ml 30% w/v sucrose cushion. The membrane band at the interface was washed three times and resuspended at a protein concentration of 3 mg ml^{-1}. The membranes were solubilised at 37 °C for 2 h following the addition of 2% w/v SDS and 2% v/v 2-mercaptoethanol. Overnight dialysis against 0.01 M phosphate buffer *p*H 7.1, containing 0.1% w/v SDS and 0.1% v/v 2-mercaptoethanol and electrophoresis on 7% w/v polyacrylamide gels in the presence of 0.2% w/v SDS was performed according to Weber and Osborn[19], 150 μg of solubilised membrane protein being applied to each gel. Glycoprotein was located by the periodic acid–Schiff reaction[20] and densitometric scanning was performed using an ISCO model UA4 absorbance monitor. The apparent molecular weights of the glycoproteins were calculated relative to the migration[7] of a series of protein standards and are shown in the figure.

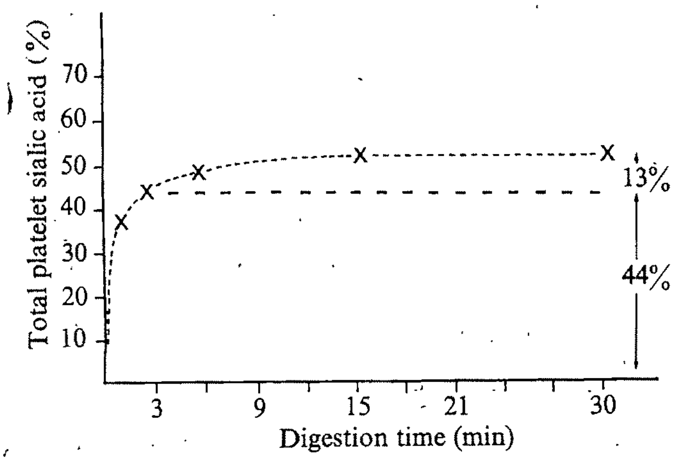

Fig. 2 The rate of release of sialic acid from washed control human platelets by trypsin. Aliquots (0.5 ml) of the supernatants obtained following the trypsin digestion of washed human platelet suspensions as described in Fig. 1 were hydrolysed for 1 h at 80 °C with 0.1 N H_2SO_4 and the sialic acid estimated by the method of Aminoff[11]. The percentage of the total platelet sialic acid (estimated as described previously[7]) released concomitant with the disappearance of the 155,000 molecular weight glycoprotein after 3 min digestion, and the disappearance of the 135,000 molecular weight glycoprotein after 15 min digestion (as shown in Fig. 1) is given.

Here we describe how the bulk of the platelet sialic acid is associated with the major platelet glycoprotein (molecular weight 155,000) and how there are specific defects in the membrane glycoproteins of platelets obtained from patients with clinically defined bleeding disorders as a result of defects in different aspects of platelet function. Human platelet membranes contain three major glycoproteins[6,7] as characterised by their rate of migration on polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS). Attempts at purifying these glycoproteins to establish their identity have been hindered by their tendency to associate following their solubilisation from the membrane[8]. Figure 1, however, shows how the membrane glycoproteins are differentially degraded during the incubation of washed suspensions of human platelets with trypsin. By calculating the amount of sialic acid released concomitant with the loss of the staining capacity of each of the glycoproteins it was shown that the 155,000 molecular weight glycoprotein contained the predominant part of the trypsin-releasable sialic acid (Fig. 2). Approximately 30 min digestion were required to remove all of the membrane glycoproteins as located by the periodic acid–Schiff reaction, and during this time the bulk of the platelet protein remained resistant to proteolytic attack. The yield of membrane protein obtained from washed platelets digested for 30 min with trypsin (5–7% of the total platelet protein) was not significantly different from that obtained from untreated platelets. Thrombin, when added to washed platelet suspensions, attacks only a limited number of sites of the intermediately migrating major membrane glycoprotein[9], and releases only 12% of the total platelet sialic acid[10].

In view of the high sialic acid content of the 155,000 molecular weight glycoprotein it would seem that this must be a much branched structure and it is probable that the mutual repulsion of the negatively charged sialic acid moieties, which only occur as the terminating monosaccharide of the glycoprotein oligosaccharide chains, gives the 155,000 molecular weight glycoprotein an extended three-dimensional configuration different from the 135,000 and the 103,000 molecular weight glycoproteins. We believe it significant, therefore, that we have been unable to locate more than traces of the 155,000 molecular weight glycoprotein in membrane fractions prepared from the platelets of two patients with the Bernard–Soulier syndrome (Fig. 3). This is a bleeding disorder associated with an impaired platelet adhesion to subendothelium[11] in spite of an apparently normal mechanism of ADP-mediated aggregation[12]. The glycoprotein abnormality is consistent with a previous report which described a reduced sialic acid content and a reduced electrophoretic mobility of the Bernard–Soulier platelets[13]. SDS electrophoresis of solubilised whole platelets confirmed the much reduced content of the 155,000 molecular weight glycoprotein and failed to locate any further gross abnormalities in the other major glycoprotein and protein constitutents. It seems to us that as a result of the probable exposed position of the 155,000 molecular weight glycoprotein that it is in a good position to act as the acceptor/receptor on the platelet surface for the macromolecular elements of the subendothelium, adhesion to which is impaired in the Bernard–Soulier syndrome[11].

This is in contrast to the apparent absence of the 135,000 molecular weight glycoprotein which we have described previously[7] in the platelets of three patients with Glanzmann's thrombasthenia, a bleeding disorder associated solely with a defective mechanism of ADP-mediated aggregation[14]. The abnormality has now been confirmed in the membranes of the platelets of a further two patients with thrombasthenia (Fig. 3), and would seem not to be associated with the first stage of the ADP aggregation mechanism as the thrombasthenic platelets respond to the initial ADP stimulus with a quantitatively normal shape change (A.T.N. and J.P.C., unpublished), a morphological shape change being the first response of the platelet to stimulation[15]. It would seem that the glycoprotein abnormality in the thrombasthenic platelets may well be manifested at a later, possibly enzymatic, stage in the aggregation mechanism. Note that Evans[16] has described a glycoprotein of a similar size, which is an enzyme associated with nucleotide metabolism in the membranes of hepatocyte cells.

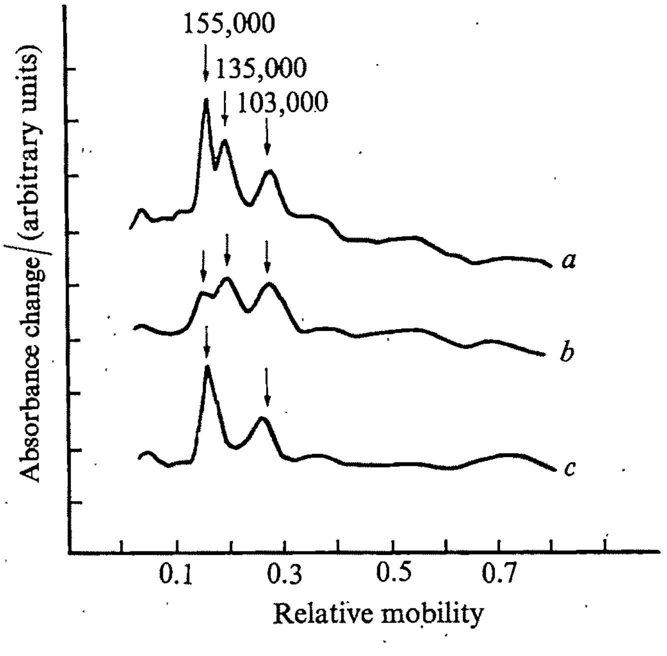

Fig. 3 Polyacrylamide gel electrophoresis in the presence of SDS showing the distribution of glycoprotein in membranes obtained from control human platelets (*a*); Bernard–Soulier platelets (*b*) and thrombasthenic platelets (*c*). Experimental procedures are as described in the legend to Fig. 1. Solubilised membrane protein (150 µg) was applied to each gel.

It would seem therefore that the layer of bound carbohydrate first shown to be present at the surface of the platelet by electron microscopic techniques[17] contains groupings which are vital to the haemostatic function of the platelet. It is interesting that differences in the glycoprotein composition of the membranes of two strains of amoebae, one of which clumps during exponential growth, have recently been described[18]. Evidence for a general role for specific membrane glycoproteins in the mechanism of cell adhesion and possibly aggregation seems to be accumulating.

This work was supported by grants from INSERM, CNRS and the British Heart Foundation.

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Effect of cholesterol on the non-electrolyte permeability of planar lecithin membranes

IT is well known that the addition of cholesterol to artificial lipid membranes decreases their permeability. Most of the studies on artificial systems have been performed on liposomes[1-4], whereas the effect of the cholesterol on planar lipid bilayers was investigated on water transport only. In particular Finkelstein and Cass[5] found that addition of cholesterol to membrane of lecithin resulted in a decrease of water permeability. In the present work we studied the effect of cholesterol addition on the non-electrolyte permeability of lecithin planar membranes. The artificial membranes were formed with egg lecithin dissolved in decane to form a 0.5% solution. Lecithin was extracted from egg yolks[6] and purified by alumina chromatography. Cholesterol, twice crystallised, was added to lecithin solution to obtain cholesterol–lecithin molar ratios of 1:1 or 2:1.

The membranes were formed at 28 °C by brushing the solution on a small circular aperture (0.07 cm^2) of a Teflon cell. The cell was similar to that described by Laüger *et al.*[7]. The hole of the cell was prepainted with membrane solution under nitrogen[8]. The bathing fluid was a 10 mM KCl solution. The non-electrolyte permeability was measured as follows. When membrane became "black" about 10 µl of water, containing about 10 µCi of a labelled molecule were added to one side, and the same quantity of unlabelled solution was added to the opposite side. After 40 min a small amount of fluid from both compartments was collected. Stirring of the solutions was achieved by convection currents provided by small temperature differences between the bottom and the top of the chambers. The labelled molecules we used were: ^{14}C urea, ^{14}C thiourea, 1-^{14}C glycerol, N-N'dimethyl-^{35}S-thiourea (Radiochemical Centre); ^{14}C acetamide, N-^{14}C methylurea (New England Nuclear); ^{14}C formamide, N-dimethyl-carbonyl-^{14}C formamide (Mallinckrodt Nuclear). The radioactivity readings were performed with a Tri Carb (3320–01) Packard liquid scintillation spectrometer and the permeability coefficients (P) calculated.

Table 1 Permeability coefficients (P) of non-electrolytes across lipid bilayer membranes

Molecule	Membranes of lecithin in *n*-decane; $P = 10^{-6}$ cm s^{-1} (Mean ± s.e.)	Membranes of lecithin cholesterol (1:1) in *n*-decane; $P = 10^{-6}$ cm s^{-1} (Mean ± s.e.)
Glycerol	7.4 ± 0.8 (6)	11.9 ± 2.6 (5)
Urea	3.5 ± 0.5 (12)	3.6 ± 0.5 (13)
N-methylurea	39.2 ± 7.6 (6)	31.4 ± 6.6 (7)
Thiourea	4.3 ± 0.5 (12)	6.3 ± 1.2 (16)
Acetamide	145.1 ± 28.5 (10)	47.8 ± 7.6 (11)
Formamide	146.4 ± 27.8 (11)	67.4 ± 14.0 (6)
N-N'-dimethyl-thiourea	186.7 ± 29.0 (10)	45.5 ± 13.4 (7)
N-dimethyl-formamide	113.4 ± 17.2 (7)	74.7 ± 11.1 (6)

Number of experiments in parentheses.

It can be seen (Table 1) that addition of cholesterol to lecithin membranes (1:1 molar ratio in the membrane solution) does not affect equally the permeability of all the tested molecules. In particular the permeability of the more permeable molecules, that is, formamide, acetamide, N-dimethyl-formamide, N-N'-dimethyl-thiourea is lowered: on the contrary molecules showing a low permeability coefficient through lecithin films that is glycerol, urea and thiourea are completely insensitive to the addition of cholesterol. A similar phenomenon has been observed in biological membranes and proteins have been invoked to explain this selectivity[9]. It is instructive to see, however, that the lipid moiety of simple model membrane may account for this selectivity also.

It is interesting that selectivity phenomena were observed by Cohen and Bangham[10] in the permeation of non-electrolytes through liposome membranes.

The permeability of N-methylurea, showing an intermediate value between the two groups of molecules is scarcely affected by the cholesterol addition. Only an increase of the cholesterol–lecithin ratio in the membrane solution (Table 2) results in a significant decrease of thiourea and methylurea permeabilities.

We can regard the nature of this selectivity displayed by the thin lipid membranes as a process in which at least three rate constants are involved in the permeation of the solutes[11,12]. Accordingly, in the overall process of permeability we can assume that $K_1 = K_3$ are the rate constants at the interface (water–membane, membrane–water) and K_2 refers to the hydrocarbon core of the membrane. According to this, K_2 is greater then $K_1 = K_3$ for molecules like formamide, whereas this difference is less marked for urea, glycerol and thiourea. The discrepancy between the effect of cholesterol on the permeability of the two groups of molecules may be explained assuming that cholesterol exerts its condensing effect mainly on the membrane core and its presence scarcely effects the interface. In other words the presence of cholesterol results in a marked decrease of K_2 for molecules like N-N'-dimethyl-thiourea, whereas its effect is relatively small for molecules like urea, whose K_2 value is low. But a further increase of cholesterol content in membrane solution seems to affect the K_2 value of thiourea and N-methylurea.

Evidently the cholesterol concentration in the membrane reaches such a value that affects the membrane structure by making it more compact. In these conditions the membrane permeability approaches that of oxidised cholesterol ones,

Table 2 Permeability coefficients (P) of non-electrolytes across lipid bilayer membranes.

Molecule	Membranes of lecithin cholesterol (1:2) in decane $P = 10^{-6}$ cm s^{-1} Mean ± s.e.)
Thiourea	2.4 ± 0.3 (8)
N-methylurea	11.7 ± 3.6 (7)

Number of experiments in parentheses.

which show a marked reduction of permeability as compared with that of lecithin films[11].

A different pattern is, however, shown by cholesterol on various artificial membranes. In fact if we compare our results on planar lipid membranes to that obtained on liposomes[1-4] we observe that the lecithin liposomes are more sensitive to the addition of cholesterol. This different effect of the cholesterol could be due to the presence of decane in lipid films. The solvent molecule could lower the cholesterol–lecithin ratio in the membrane by decreasing the possibility of interaction between these molecules. We have no evidence supporting this idea, but Montal and Mueller[13] have observed some differences between planar lipid bilayers made with and without hydrocarbon solvent.

On the other hand, Hauser *et al.*[14] have observed that the presence of defected liposomes seriously affects the exact estimation of their permeability. It is possible that cholesterol, by making the liposome structure more compact, reduces the number of defected liposomes, and consequently their permeability is more affected as compared with planar lipid membranes.

This work was supported by a research grant from CNR, Rome. We thank Professor P. Laüger and Dr U. Hopfer for advice and criticism.

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Received April 1; accepted May 12, 1975.

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Sodium transport and lithium movements across the insect blood–brain barrier

INSECTS are the only invertebrate animals which have been shown to possess a well developed blood–brain barrier. This barrier limits the rate of intercellular diffusion of water-soluble substances between the blood and the fluid layer which is the immediate environment of the nerve cells (see ref. 1). It has been proposed that this limitation results from the presence of intercellular occlusions, notably tight junctions, in the cellular layer of the nerve sheath, the perineurium (Fig. 1).

Insects also seem to be unique in their ability to regulate the ionic composition of the nerve cell environment. It has frequently been postulated that this ionic homeostasis results from active transport by the perineurium and underlying glial elements (Fig. 1). In particular, it has been suggested that sodium ions are transported from the blood to the fluid layer at the neuronal surfaces to maintain the action potentials. We present evidence which accords with this hypothesis.

We have used action potential amplitude as an indicator of extra-axonal sodium concentration in isolated central nervous connectives of the cockroach *Periplaneta americana*. The rates of change in concentration were calculated in different experimental conditions with intact preparations and with those in which the perineurium was surgically removed (desheathed).

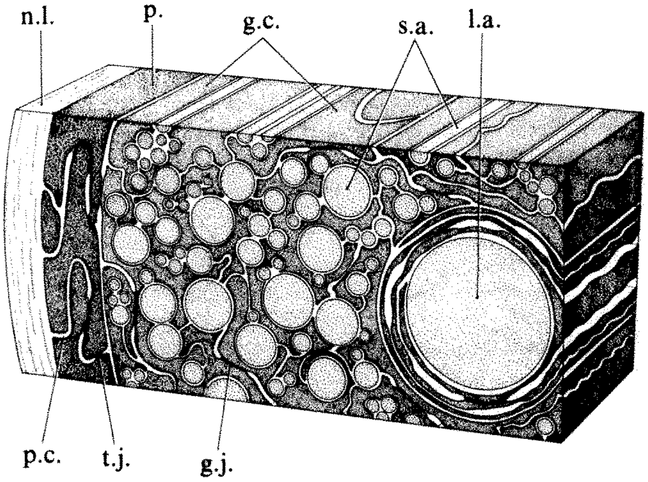

Fig. 1 Schematic view of a portion of the periphery of a cockroach central nervous connective. The superficial connective tissue sheath, the neural lamella, overlies the cellular perineurium. Beneath, the axons are enveloped, to varying degrees, by glial processes. Intercellular movements of ions and molecules through the perineurium are restricted by junctions in the perineurium[2-4]. In addition to the tight junctions invariably present at the inner ends of the clefts, there are occasional, more peripherally located, septate desmosomes. Glial elements are linked to one another, and adjacent perineurial cells, by gap junctions. n.l., Neural lamella; p., perineurium; g.c., glial cells; s.a., small axons; l.a., large axon; p.c., perineurial cleft; t.j., tight junction; g.j., gap junction.

Figure 2 shows the effects of exposure of an intact preparation to sodium-deficient (Tris-substituted) Ringer. The decline in amplitude of the action potentials, and the loss of sodium indicated by this, are slow compared with the rapid recoveries obtained on return to normal Ringer. This asymmetry, which is absent in desheathed preparations, accords with the above concept of an inwardly-directed transport of sodium ions. The rates of decline varied in different preparations, whereas the rates of subsequent recovery were remarkably constant. The following experiments describe factors which affect this recovery rate.

In spite of the ability of lithium ions to substitute for those of sodium in axonal conductance in desheathed preparations, results were obtained in which no recovery in the amplitude of the action potentials occurred when sodium-depleted connectives were exposed to Ringer in which sodium was replaced by lithium (Fig. 3). A rapid recovery was observed, however, in lithium Ringer in preparations exposed briefly to hypertonic urea solution, a procedure which is known to by-pass the peripheral blood–brain barrier[6]. The specificity for sodium is unlikely to result from passive permeability properties of the system[3] and again suggests that there is an inwardly-directed transport of sodium ions to the extra-axonal fluid. The slow recovery of the action potentials on return to normal saline (Fig. 3) could result from the effects of lithium on sodium pumping or (in view of the loss of sodium from connectives being greater in lithium than in Tris-substituted Ringer[8]) from a delay involved in re-establishing the levels of sodium available to a pump.

Externally-applied amiloride (1 mM) and ouabain (10 mM), which is known to affect the axonal sodium pump[7], were found to be without effect on the rate of recovery of the action potentials in intact, sodium-depleted connectives. The rate of recovery was, however, reduced in the presence of 2-4-dinitrophenol, which at 50 μM did not affect axonal responses in desheathed preparations. In spite of its lack of effect on the axonal sodium pump[7] the sodium-transport inhibitor, ethacrynic acid, reduced the apparent movement of sodium ions to the axonal surfaces (Fig. 4). It can be seen that this inhibitor also seemed to reduce the outward movement of sodium ions.

These effects of ethacrynic acid accord with previous observations on cockroach connectives which showed that, although the axonal sodium pump is insensitive, extra-axonal elements are affected by this compound[7]. The lack of effect of ouabain on sodium movements between the external medium and the extra-axonal fluid accords with the apparent insensitivity of the glia to cardiac glycosides as compared with the axons whose sodium pump has been shown to be inhibited by strophanthidin[7].

A previous study has shown that lithium accumulates in intact connectives to levels similar to those of sodium, and it was suggested that this accumulation occurred in the glial and perineurial elements[8] which are linked by gap junctions (Fig. 1). This suggestion is further supported by the apparent inability, reported here, of lithium to gain access to the axonal surfaces. In the presence of effective intercellular occlusions in the perineurium, we must assume that lithium can cross the outwardly-facing perineurial membranes. Since the latter have a relatively low passive permeability to lithium and sodium[3], these cations are unlikely to enter the perineurial cells by passive diffusion down an electrochemical gradient. The inability of amiloride to block the entry of sodium, and the previous observation of a retention of lithium ions during prolonged washing in normal Ringer[8], suggest that these cations could share a peripheral, unidirectional, transport system. The exclusion of lithium ions from the fluid bathing the axon surfaces would then be the result of their inability to cross the inwardly-directed perineurial and glial membranes. This inability contrasts with the ready access of sodium ions to the axon surfaces (Fig. 2) and suggests that sodium is transported across the glial membranes by a conventional pump which, as in crustacean neurones[9], will not accept lithium ions. Such a pump in this position could also support axonal function by transporting potassium ions away from the extra-axonal fluid.

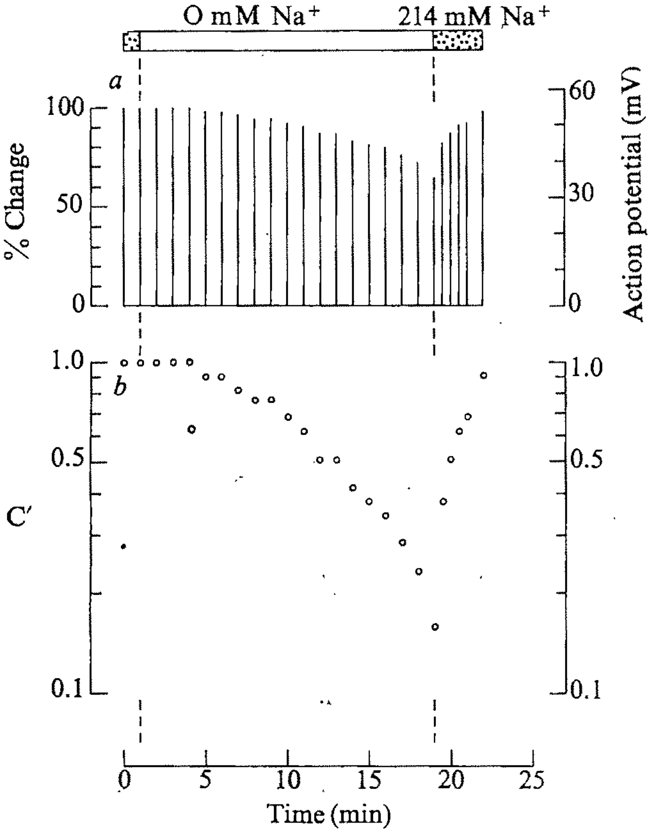

Fig. 2 *a*, Effect of sodium-deficient (Tris-substituted) Ringer on action potential amplitude. In this and subsequent figures, the amplitude of compound action potentials is shown, as recorded with the 'sucrose-gap' technique in cockroach central nervous connective, following electrical stimulation at regular intervals. Nerve cords were isolated and maintained in the high-sodium Ringer of Yamasaki and Narahashi[6] until a constant action potential amplitude was obtained before the experiments were begun. *b*, Change in relative sodium concentration at the axonal surfaces (C′) estimated from percentage change in action potential amplitude shown in (*a*). Initial extra-axonal sodium concentration is unknown and has been designated as 1.0. The change in sodium concentration relative to this value was estimated from the Nernst relationship between sodium concentration and the percentage change in action potential amplitude of surgically-exposed axons in desheathed preparations.

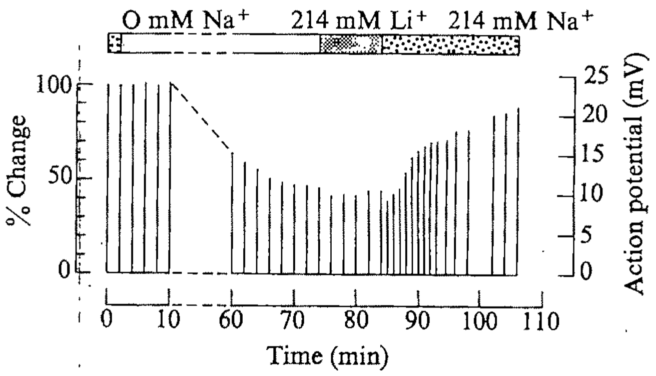

Fig. 3 Effect of exposure to lithium Ringer, following sodium depletion in the sodium-deficient (Tris) Ringer. Subsequent exposure to the original sodium concentration results in a relatively slow recovery of action potential amplitude.

The net inward movements of sodium ions between the external medium and the fluid bathing the axon surfaces therefore seem to be largely mediated by active transport through the perineurial and glial membranes—being inhibited by dinitrophenol and ethacrynic acid. Transport across the

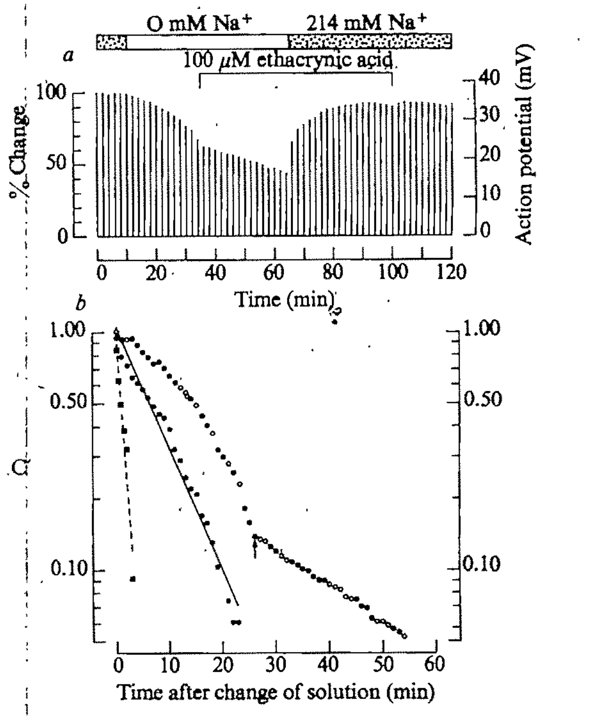

Fig. 4 *a*, Effect of 100 μM ethacrynic acid on the change in action potential amplitude during and after exposure to sodium-deficient (Tris) Ringer. *b*, Change in relative extra-axonal sodium concentration (C′) estimated from (*a*), as in Fig. 2*b*, showing a slowing of the rate of decline (○) following addition of ethacrynic acid (arrow). Recovery rate, plotted as 1−C′, in the presence of ethacrynic acid (●) is slower than that observed in an untreated preparation (■). The latter data are taken from the experiment illustrated in Fig. 2.

outer perineurial membranes seems to be effected by a pump which also accepts lithium, and across the inwardly-directed membranes by one which is specific for sodium ions.

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Received March 26; accepted May 6, 1975.

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Limit of fish swimming speed

OBSERVATION and analysis of the swimming of marine fish in laboratory aquaria and in the vicinity of fishing gear (C.S.W., unpublished) have indicated that small fish (0.1 m) can reach speeds up to 25 body lengths s^{-1} whereas larger fish (1 m) seem unable to exceed 4 body lengths s^{-1}. Bainbridge[1] showed that smaller fish were capable of higher tail beat frequencies than larger fish and forward motion resulting from one complete tail beat cycle (or one 'stride') was between 0.6 and 0.8 times the length of the fish. In the present study the tail beat frequency or frequency of strides is considered to be limited by the contraction time of the swimming muscle of the fish, and maximum observed swimming speeds are found to fit the dimensions of the muscle twitch time and stride length.

The twitch contraction time of white lateral muscle (taken from freshly killed marine teleosts, previously adapted to and rested in marine aquaria) was measured by electrically stimulating pieces of their muscle in a temperature-controlled isosmotic bath.

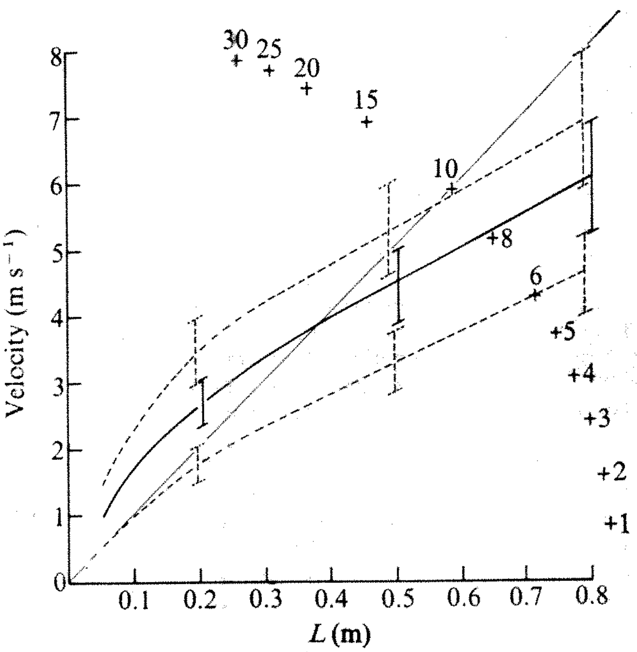

Fig. 2 Maximum swimming speeds predicted by the three muscle contraction time curves in Fig. 1 for a stride length of 0.7 times L ●: top, 20 °C; middle, 14 °C; bottom, 0 °C. Increase in speed as a result of altering A between 0.6 and 0.8 is indicated by the vertical bars. Third curved scale allows conversion of any point on the graph to body lengths s^{-1} by lining up that point with the origin of the graph. A 10 body length s^{-1} line is drawn in.

The time from the stimulating pulse to the peak of contraction was measured for a number of species of marine fish of a wide range of sizes (Fig. 1). From these individual points a continuous line representing the shortest contraction time for each size of fish was drawn. It was then assumed that the shortest time for a complete tail beat cycle or stride was limited by the muscle twitch contraction time of the muscle; for example, a contraction time of 0.04 s for muscle from a fish 53 cm long (Fig. 1) would allow 25 contractions, alternated between the two sets of opposing lateral muscles, per s and thus 12.5 complete tail beats or strides per s. With a maximum stride length of 0.8 times body length the maximum speed possible for the 53-cm fish will be $12.5 \times 0.8 = 10$ body lengths s^{-1} or 5.3 m s^{-1}. In this way the maximum swimming speed M at a stated temperature is predicted by the formula $M = AL/2T$ where L is the length of the fish (m), T is the muscle contraction time (s) and A is the stride length which according to existing measurements lies within the range 0.6–0.8 times L. The calculated maximum swimming speeds for fish with $A = 0.6$, 0.7 and 0.8 and for the shortest muscle contraction times associated with the fish lengths indicated in Fig. 1 are shown in Fig. 2.

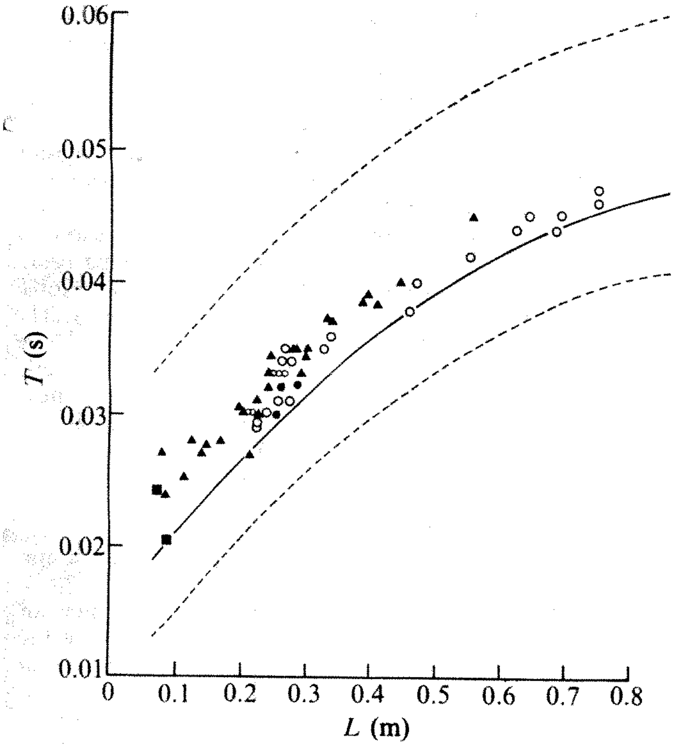

Fig. 1 Muscle contraction time for different sizes of teleost: ▲, plaice, *Pleuronectes platessa*; ○, cod, *Gadus morhua*; ■, haddock, *Melanogrammus aeglefinus*; ●, salmon, *Salmo salar*. Dashed thin curves 20 °C (bottom) and 0 °C (top) allow for a measured change in contraction time of 0.001 s for 1 °C change in temperature. The muscle block (3×1×0.5 cm) was rigidly fixed at one end in a jaw of stainless steel gauze (12 wires, 0.27 mm diameter, per cm) and was attached at the other end by a light hook to a 30 mm optical wedge isotonic transdúcer (Devices 3194), loaded with a light spring to give a tension of 20 g. Single direct current pulses (0.001 s and 20 V) were applied to the saline bath (14°C, NaCl 0.2 M) by way of stainless steel vertical plates one on either side of the muscle block. The resulting movement of the muscle was displayed on an oscilloscope, photographed and measured. Although temperature was found to have a marked effect on the time of contraction, 'after-loading' the muscle only altered the amplitude of the movement and not the time for complete contraction.

Movements of swimming fish representing a wide range of sizes of marine species were recorded on videotape using a modified closed circuit television system. Fish in conditions as described previously[2], were either startled into movement or trained to race between two feeding points by association of underwater flashing lights with the appearance of food. Maximum speeds of 26 body lengths per second were recorded for small haddock and sprats (10 cm) which show evidence of the maximum predicted tail sweep time which was close to the television frame time of 0.02 s and a stride approaching 0.9*L*; an example is shown in Fig. 3. Similarly small (seawater) salmon (25–28 cm) reached 2.5 m s^{-1} (10 body lengths s^{-1}) and the tail beat took 0.06 s for a complete cycle or 0.03 s for each muscle to contract. The highest speed for cod yet measured is 2.8 m s^{-1} for a 73-cm fish; however, a single tail-beat cycle at this speed took 0.18 s and its stride was 0.68*L*. If this cod had used its shortest predicted tail beat of 0.09 s and had maintained the same stride it would have reached 5.5 m s^{-1}. The race track between the flashing lights in the Aberdeen tank is 8 m and it is likely that this large fish weighing 3.78 kg would not achieve its maximum speed in such confined surroundings.

Using the same stride length this cod may from Fig. 2 be expected to reach 6.6 m s^{-1} at 20 °C and only 4.3 m s^{-1} at 0 °C. It seems that the shorter contraction times predicted here of the muscle of tropical fish above 20 °C may help to explain the remarkable records of the barracuda[3], *Sphyraena barracuda* (Walbaum), swimming at 12.2 m s^{-1} (L=1.22 m), the yellowfin tuna[4], *Thunnus albacares* (Bonnatare), at 20.8 m s^{-1} (L=0.98 m) and wahoo[4], *Acanthocybium solandri* (Cuvier) at 21.4 m s^{-1} (L=1.13 m), but it will be essential to measure muscle properties at known temperatures and stride lengths for these species if they are to be drawn into the same scheme. It is clear that to make use of the shorter contraction time in the larger fish the muscle must be supported by increased power and as Hill[5] and Close[6] have pointed out, in discussing muscle contraction speeds in relation to animal size, the strength of the supporting framework is critically matched to these muscle properties.

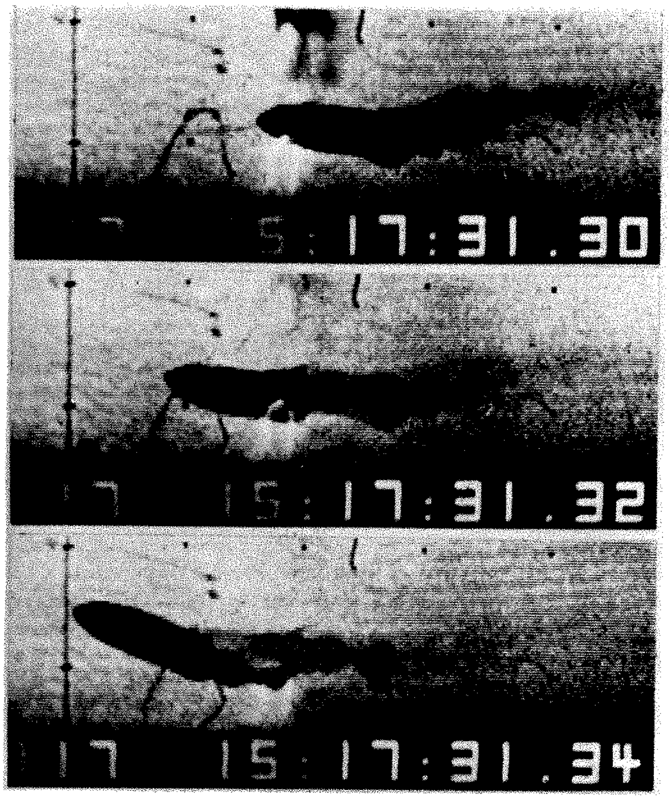

Fig. 3 Startled haddock (10 cm, 12 °C) moving at 2.5 ms^{-1}: black dots are 5 cm apart. Three photographs of single television frames are 0.02 s apart. First three digits indicate experiment number, the remaining indicate time. Note the tail is to the right of the fish's body at 0.30, left at 0.32 and right again at 0.34 s. A fish this size has a muscle twitch contraction time of about 0.02 s, approximately matching the television frame rate. Television camera was fitted with a silicon-diode array vidicon tube having high-red sensitivity and short-image retention time. Fish were illuminated with a strobed (10 µs flash), red light source synchronised to the television scan rate and placed next to the camera lens to create frozen silhouettes of moving fish against marked screens of reflex reflector material (3M Scotchlite, 3270).

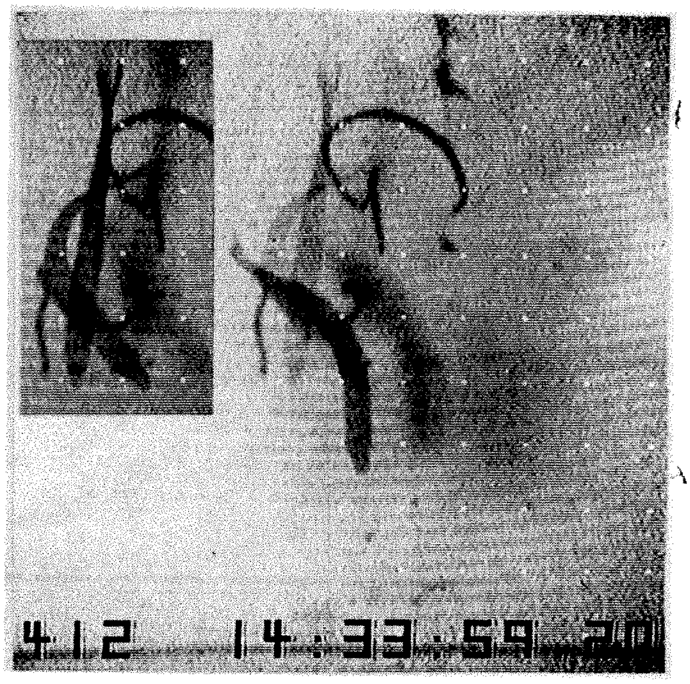

Fig. 4 Two frames 0.02 s apart show three positions of a sprat, *Sprattus sprattus* (L.) (8.3 cm, 12 °C). Frame 1 (inset), position 1, time 0.16 s (not indicated) fish is standing still; frame 1, position 2, time 0.18 s tail bent to left; frame 2, position 3, time 0.20 s, tail to right and forward speed is 1.4 m s^{-1}. Note the muscle twitch speed of an 8.3-cm fish is near 0.02 s. Television system as in Fig. 3.

It seems that the fish examined here are tailored to supply sufficient power to make full use of their shortest muscle contraction time even when fully loaded. Three frozen images at 0.02 s intervals (Fig. 4), show a sprat (8.3 cm) starting from zero speed which by the third image has travelled forwards at 1.4 m s^{-1}. Each of the opposing muscles even with the load involved in the acceleration have contracted within 0.02 s; note how the loaded single power beat (between positions 2 and 3, Fig. 4) shows a smaller amplitude than the first unloaded positioning bend.

That the initial performance of the fish's rested anaerobic muscle is exceptional, is confirmed by its biochemistry. The glycogen concentration can be as high as 1 g per 100 g muscle: 1 g glycogen releases 558 J when converted to lactic acid. The maximum rate of oxygen uptake in fish is near 1.6 mg oxygen per 100 g per min; limiting aerobic energy release to no more than 36 J per 100 g per min or 0.6 W per 100 g fish weight. Ten per cent of this yield is committed to maintenance[7]. Half of the glycogen fuel store was converted to lactic acid within the first 2 min of severe activity in rainbow trout[8], *Salmo gairdneri*. Artificial stimulation of loaded isometric plaice muscle (0.001 s, 20 V pulses, 0.02 s apart) has shown complete depletion of glycogen within 30 s. The same pulses applied singly in the same conditions resulted in a 50% drop in the twitch tension after 130 pulses and 80% tension loss after 300 pulses. Glycogen was lost at a similar rate (C.S.W., unpublished). It is clear that the rested fish could use its full contraction power together with its shortest contraction time to attain maximum speed, but the power to use its shortest contraction time will be lost quickly. These short periods at maximum speed are, however, significant in terms of survival.

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Received March 10; accepted May 19, 1975.

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Moulting hormone production in the isolated larval abdomen of the Colorado beetle

In spite of the overwhelming evidence for the essential roles of the prothoracic glands in the synthesis of moulting hormone and the regulation of insect moulting and development[1-3], several instances of moulting without prothoracic glands have been reported, either by extirpation (*Galleria mellonella*[4] and *Periplaneta americana*[5]) or by ligation (*Tenebrio molitor*[6] and *Dermestes vulpinus*[7]). Little experimental evidence is available to elucidate the significance of this phenomenon. We report here a similar moulting phenomenon observed in the Colorado beetle, *Leptinotarsa decemlineata* (Say), and demonstrate that the isolated abdomen of this species can produce moulting hormone and moult in the absence of the prothoracic glands.

Mature larvae of the Colorado beetle dig into soil and enter the prepupal stage when feeding stops. When these larvae are prevented from digging, pupation is delayed[8]. Before digging occurs, moulting is promoted by ligation between the head and the thorax, even among young larvae[9,10]. Furthermore, when the ligature is placed between the thorax and the abdomen, the isolated abdomen can moult successively into a pupal and an adult abdomen. The moulting of the isolated abdomen can be obtained in feeding larvae as young as 1.5 d after the last larval moult.

The two most likely hypotheses to explain the nature of this unusual moulting of the isolated abdomen are: sufficient moulting hormone is already present at the time of ligation and persists afterwards; or the moulting hormone is produced by the tissues to induce a moult. We investigated these possibilities by first determining the moulting hormone titres of larval and pupal stages by means of chemical extraction[11] and the *Musca* test[12].

The Colorado beetles were reared on potato leaves at 25 °C and a photoperiod of L:D 18:6. In these conditions, the last larval instar requires 8 d (3.5 d feeding stage followed by 4.5 d prepupal stage) and the pupal stage lasts for 6 d. We found no detectable amount of moulting hormone (less than 0.5 *Musca* unit (MU) per gram fresh weight; 1 MU = 3.5 ng of ecdysterone in our test) during the entire last larval feeding stage and the first day of the prepupal stage. Moulting hormone was detected in 2-d-old prepupae and reached a peak of 30 MU g^{-1} in 3-d-old prepupae. It began to decline in 3.5-d-old prepupae and fell to a low level shortly before the larval–pupal moult. In the pupa, moulting hormone activity began to increase during the first day and reached a peak of 100 MU g^{-1} on the third day. The titre again declined on the fourth day and became undetectable 1 d before the adult emerged. Our results clearly demonstrate that before or at cessation of feeding, the larva does not contain a detectable amount of moulting hormone in its tissues. Therefore, the most feasible explanation seems to be the synthesis of moulting hormone by the isolated abdomen.

The ligated abdomen of a mature larva requires about 10 d to moult compared with 4.5 d needed for a normal larva. By injecting graded doses of ecdysterone (0.1–1.0 μg per abdomen), we can gradually shorten the time before moulting, to the normal duration. This result suggests that the delay of moulting in the isolated abdomen may be the result of a slow rate of synthesis or an insufficient amount of moulting hormone in the tissues. We then proceeded to extract moulting hormone from abdomens 1 and 7 d after ligation. We found no detectable amount after 1 d, but obtained an average of 10 MU g^{-1} of moulting hormone in abdomen 7 d after isolation. The above data prove conclusively that the isolated abdomen of the Colorado beetle is capable of producing sufficient moulting hormone to induce a moult.

The significance of moulting hormone production outside the prothoracic glands is now becoming more evident. Several findings have led us to conclude that in the pupal stage of the Colorado beetle, moulting hormone is produced by the tissues rather than by the prothoracic glands. First, we have observed in this species, as others had reported in the case of *T. molitor*[13,14], that the prothoracic glands degenerate soon after the larval–pupal moult, although the highest titre of moulting hormone we detected occurred in the 3-d-old pupa. Second, when newly moulted pupae were ligated between the thorax and the abdomen, we found no difference in the time of the adult moult between the anterior and the posterior portions, indicating that the presence of the neuroendocrine systems is not essential for adult development. Our finding of actual moulting hormone production in the isolated larval abdomen also strongly suggests that a similar mechanism exists in the pupal tissues. This evidence does not exclude the essential role of the prothoracic glands during the larval–larval and larval–pupal moults. By a series of ligation experiments with larvae and prepupae of different age, we established a critical period after which ligature between the thorax and the abdomen no longer influences the timing of the larval–pupal moult, indicating that the prothoracic glands are functional in normal insects. Our results, however, suggest that tissue synthesis of moulting hormone supplements that of the prothoracic glands.

The ability of the isolated larval abdomen to convert isotopically labelled cholesterol into ecdysones has been demonstrated in two species of Lepidoptera, *Bombyx mori*[15] and *Mamestra brassicae*[16]. As regards the site of moulting hormone synthesis outside the prothoracic glands, the oenocytes have often been suggested, as in *Calpodes ethlius*[17] and in *T. molitor*[18]. Romer *et al.*[3] showed that the oenocytes of *T. molitor* larvae can convert ^{14}C-cholesterol into ecdysterone *in vitro* and concluded that moulting hormone is synthesised by the oenocytes in both larvae and pupae. Whether the oenocytes or other tissues are responsible for the production of moulting hormone in the isolated larval abdomen of the Colorado beetle remains to be demonstrated. The chemical nature of the moulting hormone and the mechanisms by which tissue synthesis is regulated also require further elucidation.

We thank Dr W. Mordue of Imperial College for comments. This work was supported in part by a research fellowship (to T.H.H.) from Agricultural University, Wageningen, Netherlands.

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Use of an X-ray television for diffraction of the frog striated muscle

THE exposure time needed to record low angle diffraction patterns from biological materials can be shortened to a large extent by using a sensitive detector instead of X-ray films. One such detector, a position sensitive proportional counter, has proved useful in studying the kinetics of the structural transitions of a lipid membrane[1] and a photoreceptive membrane[2]. We have tried another type of fast detector, an X-ray television, to study the low-angle equatorial diffraction patterns from the frog striated muscle during contraction.

The X-ray television[3] consisted of a phosphor screen viewed from behind by an image orthicon tube. The diffraction pattern formed on the phosphor screen was displayed on a TV monitor, and was photographed with high speed film (Kodak Tri-X). The spatial resolution of this detector system was 150 µm on the surface of the phosphor screen.

The sartorius muscle was dissected from a bull frog, *Rana catesbeiana*. The muscle was mounted in a Perspex chamber filled with Ringer's solution (0 °C). The proximal end of the muscle was fixed in the chamber by clamping the pelvic bone with a double hook. The distal end was connected to a tension transducer. The sarcomere length of each specimen was adjusted to 2.2 µm using the diffraction of a laser light (λ=632.8 nm).

Figure 1*a* shows a densitometer trace (right half only) of the equatorial pattern of a resting muscle recorded by the TV detector. Two reflections are clearly seen, and are indexed 1,0 and 1,1 from the hexagonal filament lattice[4]. The exposure time needed was 10 s. When X-ray film (Sakura N) was placed in the position of the phosphor screen, an exposure of about 15 min was needed to record the same reflections. Thus the TV detector was about 90 times faster than the film.

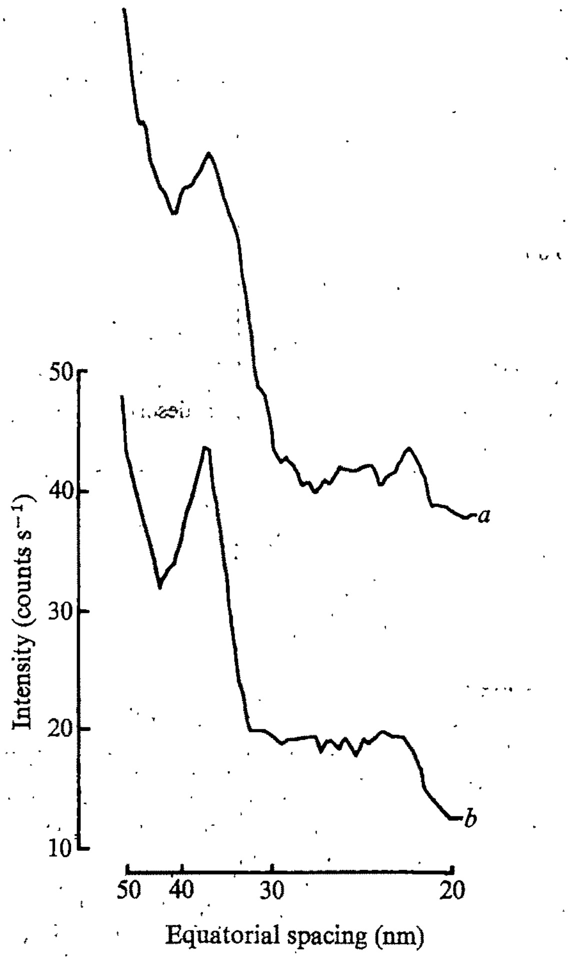

Fig. 1 *a*, A densitometer trace of the equatorial reflections recorded with an X-ray television from a resting frog sartorius muscle at a sarcomere length of 2.2 µm. The muscle was suspended in Ringer's solution (115 mM, NaCl; 2.5 mM, KCl; 1.8 mM, $CaCl_2$; 66.7 mM, dextrose; 2.15 mM, Na_2HPO_4; 0.85 mM, NaH_2PO_4; *p*H = 7.2), and was exposed to X rays for 10 s with a specimen-to-detector distance of 75 cm. The X-ray generator was a rotating-anode type (Rigaku RU100) with a line focus (1 × 0.1 mm) on a copper target. This was operated at 40 kV with a tube current of 35 mA. A low-angle camera of Franks type was used to collimate X rays. The TV detector was operated in the integrating scan mode; the scanning beam was switched off during the 10–s exposure so that the diffraction pattern was integrated on the tube target. After the exposure, the integrated pattern was read on several TV frames by switching on the scanning beam. The 1,0 reflection (the peak at the left) was stronger than the 1,1 reflection (the peak at the right). *b*. Intensity distribution of the equatorial reflections obtained from the same specimen using a scintillation counter placed behind a narrow slit (100 µm wide). Each measurement took 100 s, and the slit was moved by 100 µm before each measurement.

The relative strength of the 1,0 and 1,1 reflections can be related to the state of the myosin projections[5,6]. It is of interest to know whether the TV method provides correct information about the intensity ratio ($I_{1,0}/I_{1,1}$). Therefore the intensities of the reflections were measured with a scintillation counter (Fig. 1*b*), and the ratio obtained was compared with that calculated from the densitometer trace (first row of Table 1). There was no significant difference between the two ratios.

Table 1 Average intensity ratios ($I_{1,0}/I_{1,1}$) at different physiological conditions

	TV-detector	Scintillation counter
Resting (before tetanus)	2.39±0.13 (5)	2.26±0.23 (6)
Rigor	0.36±0.11 (5)	0.31±0.04 (4)
Tetanus	0.51±0.06 (6)	
Resting (after tetanus)	1.65±0.17 (5)	

The intensity of each reflection was assessed from the densitometer trace or from the intensity measurements from a scintillation counter, by measuring the area under each peak. Values are means±s.d. Number of experiments in parentheses. In each experiment, the measurement of the area was carried out for both the left and the right half of the densitometer trace (Figs 1–3 show only the right halves of the trace).

Figure 2*a* shows a densitometer trace (right half only) of the equatorial reflections of a muscle in rigor. The 1,1 reflection became stronger than that of the resting muscle. Figure 2*b* shows the intensity measurements from the scintillation counter. The intensity ratio obtained by the TV method corresponded with that obtained from the scintillation counter (second row of Table 1).

When the living muscle was stimulated with supramaximal pulses, it developed a tension of about 2.4 kg cm⁻². Separate experiments using the light diffraction method showed that the sarcomere length of the muscle, which was fixed in the chamber as described above, shortened by

3–7% while developing the maximum tetanic tension. To compensate for this deviation from the isometric condition, the muscles for the X-ray experiments were stretched by 5% before the tetanic stimulation. The muscles produced the equatorial patterns when exposed to X rays for 10 s during tetanus, but did not sustain the maximum tension (P_0) for the length of the exposure; the tension started to decline at 5–8 s after the onset of tetanus. We used the patterns from the specimens which sustained more than 0.80 P_0 at the end of the exposure. In these "selected" specimens, the tension returned to the resting level on cessation of the stimuli. Figure 3 is a densitometer trace of the equatorial pattern thus obtained. The 1,1 reflection was intensified during tetanus; the intensity ratio (third row of Table 1) was close to that of the muscle in rigor. A Fourier analysis similar to that described by Haselgrove and Huxley[6] showed that 86–89% of the myosin projections were in the vicinity of the thin filaments during tetanus. This is a far higher percentage than that obtained by Haselgrove and Huxley[6] for contracting muscles (52–58%). A part of this difference might have been caused by the fact that we used a long tetanus whereas they used short tetani; some rigor-like links might have been formed between myosin and actin during a long tetanus, increasing the number of myosin projections around the thin filaments. The presence of such links was detected in separate experiments as follows. The muscle was set at a sarcomere length of 2.2 µm, and was tetanised for 10 s. From 10 s after the end of the tetanus, the muscle was exposed to X rays for 10 s to produce a "post-tetanus" pattern. The result (fourth row of Table 1) indicated that the intensity ratio was smaller than that observed before tetanus. A Fourier analysis showed that 25–26% of the myosin projections stayed in the vicinity of the thin filaments after the tetanus.

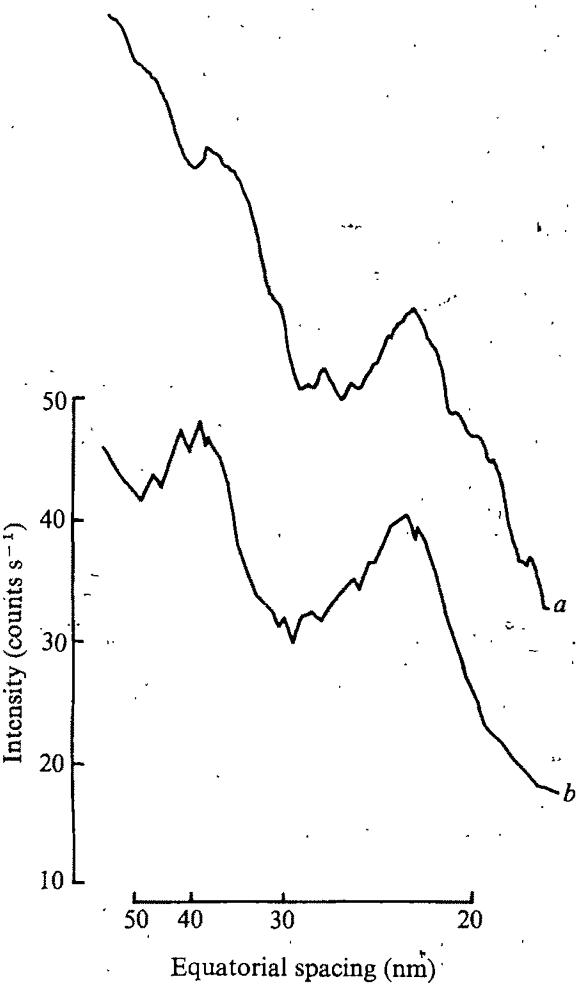

Fig. 2 *a*, A densitometer trace of the equatorial reflections recorded from a muscle in rigor at a sarcomere length of 2.2 µm. The muscle was put into rigor by soaking it for a day in Ringer's solution containing 1 mM iodoacetate. The conditions for recording were the same as in Fig. 1*a*. The 1,1 reflection was stronger than the 1,0 reflection. Note that the 1,0 peak is broad, ranging from 50 to 30 nm. *b*, Intensity distribution obtained from the same specimen using a scintillation counter.

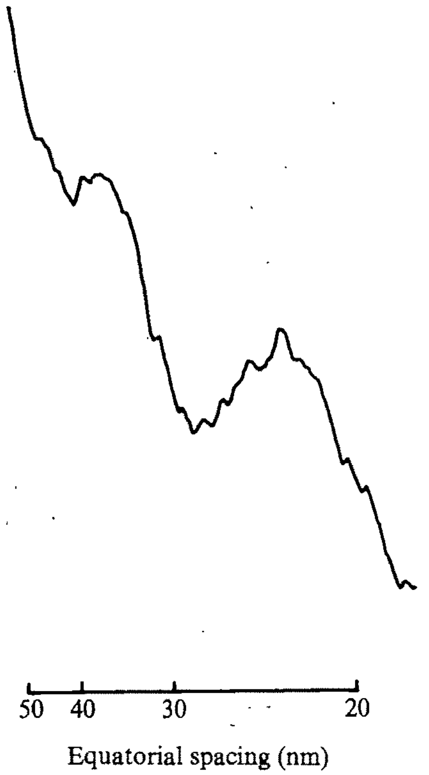

Fig. 3 The equatorial reflections from a sartorius muscle during a 10-s tetanus. The muscle was stimulated with supra-maximal rectangular pulses (1-ms duration, 100 Hz) through a pair of platinum electrodes placed parallel to the muscle axis. The muscle length had been increased by 5% before the stimulation so that the sarcomere length became approximately 2.2 µm during tetanus. The pattern resembled that of a muscle in rigor.

The presence of the rigor-like links during a prolonged tetanus, even if their fraction is very great, does not affect the conclusion that most of the myosin projections are in the vicinity of the thin filaments while undergoing cyclic reactions with actin. Let us suppose that as many as 30% (>25~26%) of the projections stay in the rigor complex throughout the 10-s tetanus. Then 70% (100–30) of the projections are undergoing the cyclic reaction, and at least 56% (86–30) are in the vicinity of the thin filaments during the reaction. This still leads us to conclude that at least 80% (56/70) of the "cycling" projections are in the vicinity of the thin filaments when the muscle is producing the maximum isometric tension.

We thank Professors K. Kohra and S. Ebashi for advice and encouragement.

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Ultrastructural demonstration and analytical application of extrajunctional receptors of denervated human and rat skeletal muscle fibres

WE describe a new approach for distinguishing denervated from normal skeletal muscle fibres, based on the visualisation of differences in distribution of acetylcholine (ACh) receptors in the sarcolemma. Iontophoretic application of ACh[1,2] and autoradiography with labelled α-bungarotoxin (αBuTX)[3,4] have shown that after denervation (and before innervation[5]) ACh receptors appear throughout the sarcolemmal membrane, in contrast to their normal location in the neuromuscular junction. Using an immunoperoxidase staining technique[6,7] to localise αBuTX, which binds specifically to ACh receptors[3,4,8], we now have a rapid and convenient method for high resolution, at light and electron microscopic levels, of the ACh receptors present throughout the sarcolemmal membrane of defectively innervated human and rat skeletal muscle fibres. We have used this immunoperoxidase method of molecular probing to analyse muscle biopsies in various human neuromuscular diseases and animal models.

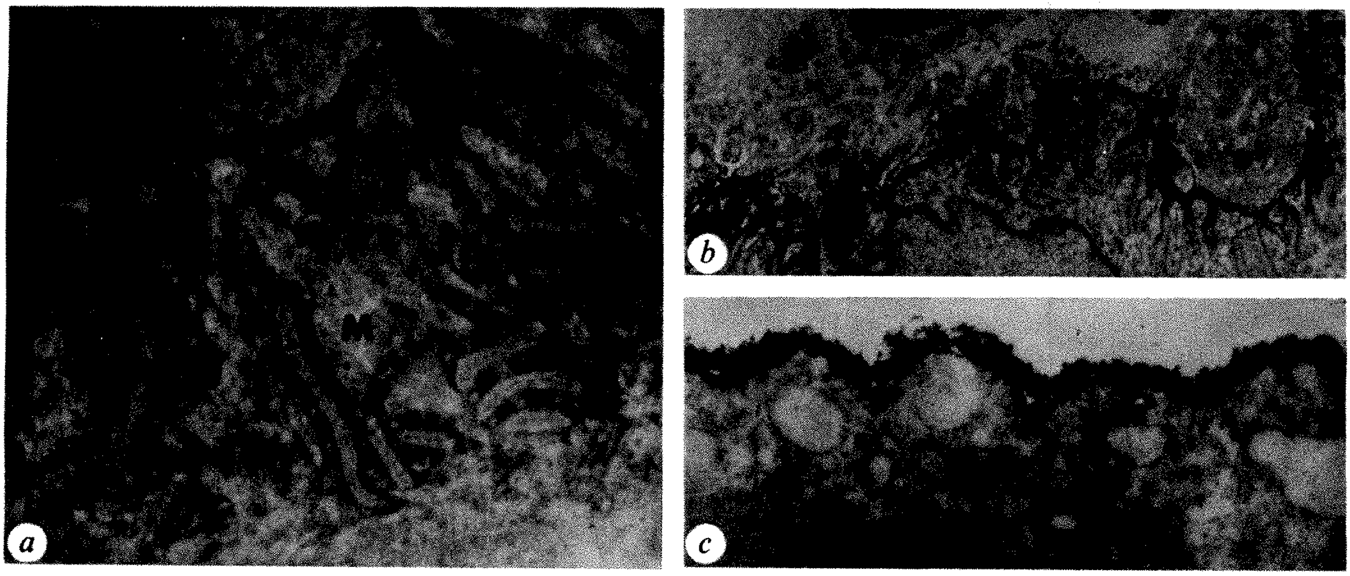

Fig. 1 ACh receptor localised by immunoperoxidase staining (black precipitate) of αBuTX binding. No counterstain was used. *a*, Normal human neuromuscular junction showing staining present at the peaks of the junctional membrane folds of the muscle fibre (M), and also on the junctional membrane of the terminal axon (Ax). (Electron micrograph ×23,500.) *b*, Normal rat diaphragm neuromuscular junction with staining similar to the human. Some staining is also present in the perinuclear membrane immediately below the junction (bottom centre). (Electron micrograph ×11,000.) *c*, Denervated rat diaphragm muscle fibre showing intense stianing of both the plasma and basement membranes. The membrane of a t-tubule running perpendicular to the plasma membrane is also stained. (Electron micrograph ×36,000.)

NIH strain rats (150–200 g) were anaesthetised with an intraperitoneal injection of sodium pentobarbital. The left phrenic nerve was hooked through a thoracic stab wound and transacted. At intervals of 2–45 d, after denervation, animals were killed and the entire diaphragm was removed and prefixed for 2 h in cold 2% paraformaldehyde in 0.1 M sodium phosphate buffer at *p*H 7.4. Fibres were teased apart and incubated at 20 °C in 5×10^{-8} M αBuTX. After fixation in a cold periodate–lysine–paraformaldehyde solution[9], they were incubated successively at 20 °C in rabbit antibody to αBuTX and peroxidase-conjugated goat anti-rabbit immunoglobulin[7] and post-fixed in cold 2% glutaraldehyde. Fibres were stained with 3,3′-diaminobenzidine (0.1 mg ml^{-1}) and H_2O_2 (0.001%) (modification of procedure of Graham and Karnovsky[10]) and treated in 1% OsO_4 for 1 h. Specimens were embedded in low viscosity Spurr or Epon 812. Sections (1 μm and 70 nm) were examined by light and electron (AEI EM 68) microscopy.

In normal human and rat skeletal muscle, staining consisted of an even precipitate sharply localised to the neuromuscular junction (Fig. 1*a* and *b*). Most of the αBuTX binding was localised on the peaks of the postjunctional folds, as described in other species[6,11]. Slight additional stain was seen in the synaptic cleft as well as in a precise deposition along the presynaptic membrane. This could have been the result of either diffusion of peroxidase reaction product or actual presynaptic αBuTX binding. If the presynaptic binding of αBuTX was not a diffusion artefact, it would agree with Koelle's proposal of ACh receptors on the presynaptic membrane of the neuromuscular junction[12] as well as being an explanation of the suggested presynaptic aspect of (+)-tubocurarine action[13]. The extrajunctional sarcolemma (that is, plasmalemma) was unstained (Fig. 1*a*), except for a scant end product within 5 μm of the junction, which may represent small numbers of ACh receptors known from ACh sensitivity studies to exist in the parajunctional sarcolemma[14]. In the rat diaphragm fibres, there was consistent but unexplained staining in the perinuclear membrane of nuclei in the immediate subjunctional region (Fig. 1*b*); intervening myofibrils, mitochondria and sarcoplasmic reticulum were not stained. The perinuclear staining was not seen in human specimens or in rat specimens with αBuTX omitted.

In denervated rat diaphragm the muscle fibres showed the progressive morphological changes known to occur with denervation[15]. The extrajunctional sarcolemma was stained sharply along the length of the fibre (Fig. 1*c*) and could be distinguished clearly from that of an unstained normally innervated fibre. This spread of receptor way seen as early as 3–4 d, reaching a maximum at 14–21 d after denervation. After 3–4 weeks, the staining intensity declined. By 40 d only scant extrajunctional staining was seen, while the former junctional region remained darkly stained. The basement membrane adjacent to the stained sarcolemma was also stained; whether or not this was caused by diffusion of end product is yet to be resolved. Occasionally, stain was located on membranes of t-tubules for a short distance after their entry into the muscle fibre,

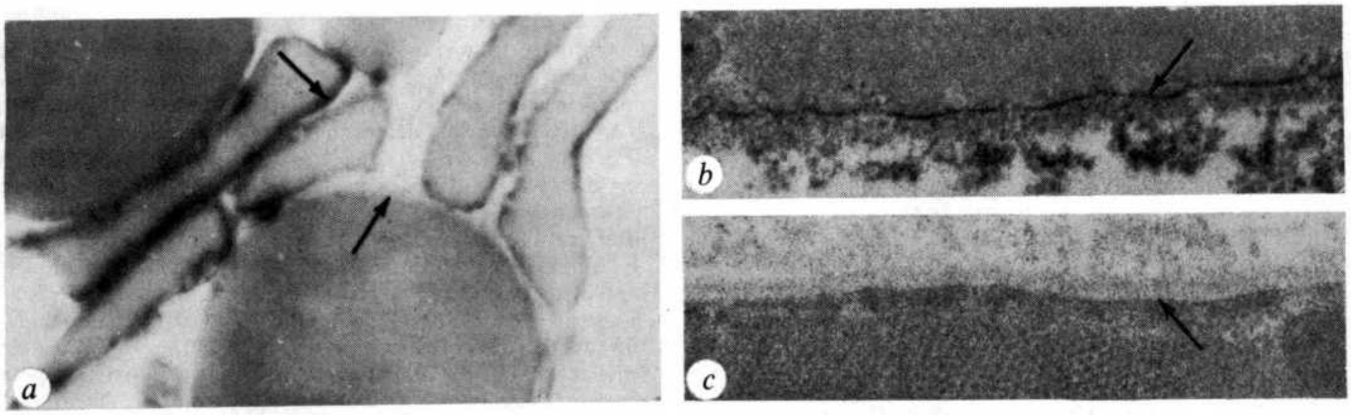

Fig. 2 Human muscle fibres in cross section from a patient with lower motor neurone disease. *a*, Group of small angular (denervated) fibres with distinct dark membrane staining (upper arrow) and two large round normal-appearing fibres with unstained membranes (lower arrow); (light micrograph ×675). Lower: periphery of one of the denervated fibres shown in (*a*). The plasma membrane (⟶) is darkly stained and the basement membrane only faintly stained. (Electron micrograph ×46,000.) *c*, Normal-appearing fibre adjacent to that in (*b*). Plasma membrane (⟶) is unstained. (Electron micrograph ×46,000.)

usually in regions of most intense sarcolemmal staining (Fig. 1*c*).

In human muscle biopsied from patients with clinical and electromyographic evidence of lower motor neurone diseases (Fig. 2*a*, *b* and *c*), staining similar to that of the denervated rat was seen throughout the sarcolemma of small fibres with angular cross-sectional contours. In serial transverse sections, these fibres were considered to be denervated according to various histochemical criteria[16]. There was some staining of the basement membrane. Extrajunctional staining was not seen on histochemically normal fibres in the same biopsies (Fig. 2*a* and *c*).

With αBuTX or either antibody omitted, no staining was seen in normal or denervated human or rat specimens.

The αBuTX immunochemical method can identify denervated skeletal muscle fibres with defective innervation and has provided us with a new approach to the analysis of human neuromuscular diseases. We are using this method to search for an ACh receptor blocking factor in sera of patients with myasthenia gravis. When we used human muscle biopsies containing either normal neuromuscular junction or denervated muscle fibres, sera of 75% of the patients showed the presence of such a blocking factor (our unpublished results). This method also provides a probe for altered innervation states in some patients with diagnostically uncertain clinical syndromes, for example, facioscapulohumoral and limb-girdle syndrome, as well as in certain unusual neuromuscular disorders generally thought to be myopathic but recently postulated to be caused by subtle defects in innervation[16].

This work was done in part during the tenure of a Research Fellowship from the Muscular Dystrophy Associations of America to S.P.R.

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Subsensitivity of noradrenaline-stimulated cyclic AMP accumulation in brain slices of *d*-amphetamine-treated mice

Both noradrenaline (NA) and dopamine are able to stimulate the synthesis of cyclic AMP in brain tissues *in vitro*[1,2]. Furthermore, several iontophoretic studies have indicated that the hyperpolarisation elicited by catecholamines in cerebellum[3], hippocampus[4], striatum[5] and cortex[6] is mediated by cyclic AMP, suggesting that this nucleotide may act as a second messenger for some actions of the catecholamines at central synapses.

It has been shown that treatments with agents like 6-hydroxydopamine[7,8] or reserpine[9], which result in an impaired stimulation of catecholamine receptors, enhanced the responsiveness of the catecholamine-sensitive adenyl cyclase of brain slices or homogenates. This effect parallels the well known electrophysiological and behavioural supersensitivity observed in such conditions[5,8].

We report here that treatment of mice with *d*-amphetamine, a drug known to induce an overstimulation of noradrenergic receptors[10], conversely results in a decreased accumulation of cyclic AMP elicited by NA in cortical slices.

Male Swiss albino mice (15–20 g; Janvier, Le Genest, France) were housed in groups of four, in a well ventilated room at an ambient température of 22 °C and under artificial illumination (light between 0800 and 2000). Food was available *ad libitum*. *d*-Amphetamine tartrate (100 μg ml^{-1}) was given in the drinking water together with saccharose (2%). Control mice were also given saccharose in the drinking water. To obtain a precise initiation of drug intake, the animals were deprived of water 1 d before the beginning of the treatment. Drug consumption was evaluated by weighing the bottles. At various times after the beginning of drug intake, the mice were stunned, decapitated

Table 1 Accumulation of cyclic AMP induced by NA in cortical slices at various time intervals during treatment with *d*-amphetamine

Duration of treatment		Basal level	NA (100 μM)	% Control stimulation
		pmol per mg protein		
0	(41)	13.9±0.7	79.6±4.3	100
5 h	(6)	17.1±1.6	83.1±5.3	76±3*
12 h	(12)	14.1±1.1	68.8±5.4	77±3†
60 h	(9)	14.0±1.3	60.5±5.5	82±4*
6 d	(17)	13.2±0.9	55.3±5.1	79±3†
10 d	(6)	10.9±1.5	47.9±6.8	71±6†

At various time intervals after treatment with *d*-amphetamine the cortical slices were incubated with or without 100 μM NA. Values are means ± s.e.m. Number of pools indicated in brackets. Control stimulation values are expressed as percentage maximal stimulation of control mice in each experiment.

$*P<0.05$ and $\dagger P<0.005$ from corresponding controls.

and their brains removed and dissected on ice. Cortical slices (250 μm thick) were prepared as described previously[11]. Usually, the slices from two to three animals were pooled together, washed and preincubated at 37 °C for 40 min in a Dubnoff metabolic shaker under a constant stream of O_2/CO_2 (95 : 5). After washing, the slices were distributed in test tubes (about 5 mg of tissue per tube), gassed and further preincubated for 10 min. The incubation medium was then supplemented with appropriate test agents, the incubation was continued and cyclic AMP determined by the protein kinase binding method[11]. Preliminary experiments had shown that NA-induced cyclic AMP accumulation was maximal between 15 and 30 min of incubation and basal level remained approximately constant until 30 min; therefore, in all further experiments, the incubations were continued for 20 min.

d-Amphetamine produced an immediate hypodipsic effect as evidenced by the water consumption during the first 12 h (1.2±0.3 ml per mouse as compared with 5.6±0.3 ml in control mice) corresponding to a drug intake of 6 mg kg^{-1}. The water consumption progressively increased, however, during the following days so that drug intake reached 9 mg kg^{-1} on day 6. The accumulation of cyclic AMP induced in cortical slices by a supramaximal concentration of NA (100 μM) was determined at various time intervals after the initiation of drug treatment (Table 1). The response to NA was already significantly smaller in slices from 5 h-treated mice and there was a slight further decrease after a 10-d treatment. Concentration–response curves for the amine were determined in mice treated with *d*-amphetamine for 6 d (Fig. 1). NA-induced accumulation of cyclic AMP in cortical slices apparently followed Michaelis kinetics as the curves are well fitted with the hyperbola of Michaelis–Menten. This allows the determination, with diverse linear plots, of apparent V_{max} and K_m. Treatment with *d*-amphetamine resulted in a significant modification of the V_{max} (847±26% in control against 578±23% in treated mice), whereas the apparent K_m was not significantly changed (1.2±0.6 μM in control against 0.8±0.4 μM in treated mice).

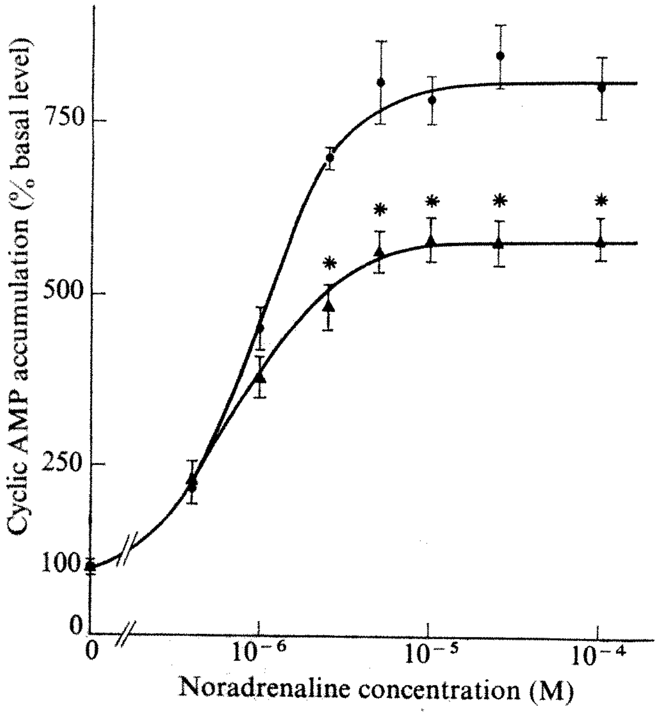

Fig. 1 Effect of noradrenaline on cyclic AMP accumulation in cortical slices of mice treated with *d*-amphetamine for 6 d. (▲) ●, Controls. Results (mean ± s.e.m.) are expressed as percentage respective basal levels (14.7±0.8 pmol per mg protein for control and 16.7±1.7 pmol per mg protein for *d*-amphetamine). Reciprocal plot of Dixon (S against S/V) was used for V_{max} determination and gave straight lines ($r=0.99$ and $r=0.99$). Eadie plot (V against V/S) was used for K_m determination ($r=0.64$ and $r=0.68$). $*P<0.01$.

To test the specificity of the effect of *d*-amphetamine treatment, the cyclic AMP accumulation induced by different agents was evaluated. Dopamine and 5-hydroxytryptamine, however, elicited only a small stimulation (Table 2) and no significant modification in responsiveness could be detected in the present conditions. In contrast, adenosine markedly activated the cyclic AMP accumulation and, after 6 d of *d*-amphetamine treatment, the response to this agent was reduced by about 25%, although this decrease was not significant.

Table 2 Accumulation of cyclic AMP elicited by various effectors in cortical slices of *d*-amphetamine-treated mice

Effector	Control (%)	*d*-Amphetamine (%)
Noradrenaline	(6) 771± 42	(9) 585± 19*
Dopamine	(6) 163± 15	(9) 154± 7
5-Hydroxytryptamine	(6) 193± 9	(9) 174± 7
Adenosine	(6) 1944±185	(9) 1551±111

$*P<0.001$.

Values are mean ± s.e.m. Number of assays is indicated in brackets. Cyclic AMP accumulation induced by various effectors (100 μM) was measured in cortical slices of mice treated with *d*-amphetamine for 6 d. Results are expressed as percentage respective basal levels: 18.4±2.1 pmol per mg protein for controls and 13.7±1.2 pmol per mg protein for *d*-amphetamine.

Thus, after treatment with *d*-amphetamine, the NA-induced accumulation of cyclic AMP in cortical slices is decreased, an effect which seems to be relatively specific to NA. Such an effect does not seem to be related to the presence of *d*-amphetamine in the slices as the addition of this drug in the incubation medium (at 1 μM) does not affect either the basal level or the NA-induced accumulation of cyclic AMP. Moreover, *d*-amphetamine elicited alterations in the mechanisms of NA inactivation (through inhibition of monoamine oxidase activity or a reuptake process) cannot account for the decreased V_{max}: they would presumably only decrease the apparent K_m by potentiating the effects of low concentrations of NA. A nonspecific alteration of phosphodiesterase activity also seems unlikely as the stimulation induced by dopamine or 5-hydroxytryptamine is not altered at a time when sensitivity to NA is modified. In the same conditions, the response to adenosine is reduced, although not significantly. This may not be totally unexpected considering that adenosine and NA interact with a common cyclic AMP-generating compartment[12,13]. It is not clear whether the subsensitivity to NA, induced by treatment of mice with *d*-amphetamine, is related to the catecholamine refractoriness observed in brain slices[14] or cultured glioma cells[15], after successive additions of the agonist, a phenomenon implicating activation of phosphodiesterase and/or rapid protein synthesis.

The NA-sensitive cyclic AMP generating system is postulated to be composed of a receptor, a transducer, a catalytic unit and, possibly, a specific phosphodiesterase[16]. The results of the present study do not allow us to determine which step in this complex model is modified by *d*-amphetamine treatment. As is the case after 6-hydroxydopamine[7] or reserpine[8] treatment, however, the modification of the sensitivity to NA is associated with a modification of the apparent V_{max} rather than with an altered K_m. This suggests that a common regulatory mechanism could be responsible for these changes, implying that the state of sensitivity of the system to NA may be modified in opposite directions according to the tonic release of the mediator.

Such a mechanism could have many implications, especially if one assumes that the sensitivity to NA of the cyclic AMP-generating system *in vitro* accurately reflects the state of responsiveness of noradrenergic postsynaptic receptors *in vivo*. First, this modified responsiveness may potentially represent a postsynaptic mechanism modulating the synaptic transmission in response to increased or decreased presynaptic activity. Such an adaptive mechanism has been already postulated to account for the modified sensitivity of the adrenergic receptor in the pineal gland, following the diurnal fluctuations of sympathetic nerve activity[17,18]. Secondly, a decreased responsiveness to NA after *d*-amphetamine treatment may be partly responsible for the 'tolerance' to some central actions of this drug. This hypothesis, which does not exclude the participation of presynaptic mechanisms such as the formation of a false mediator or the depletion of NA stores[19,20], fits well with the theoretical model of tolerance involving a modified number of receptors[21]. The unchanged responsiveness to dopamine after *d*-amphetamine treatment may tentatively be related to the absence of tolerance to some central effects of this drug. No firm conclusions, however, can be drawn in this respect considering the small stimulation elicited by dopamine as well as the low drug dosage the effects of which on cortical dopaminergic neurones[22] are not yet known. Finally, the decreased responsiveness to NA, if it persists after drug withdrawal, may well account for the post-amphetamine depression[23] and the associated psychological disturbances observed in humans[24]. Work is in progress to test this hypothesis.

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Received April 11; accepted May 14, 1975.

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Spermine inhibits induction of ornithine decarboxylase by cyclic AMP but not by dexamethasone in rat hepatoma cells

POLYAMINE biosynthesis is one of the earliest events occurring during tissue growth, and the polyamines have been implicated in the control of both RNA and protein synthesis. Increased polyamine synthesis reflects a rapid elevation of the activity of ornithine decarboxylase (ODC), the rate-limiting enzyme in the biosynthesis of the polyamines spermidine and spermine[1,2]. Fast growing tissues exhibit the highest ODC activity, whereas in slowly growing or in non-growing tissues ODC activity is very low. ODC has been induced by various agents that affect growth processes, such as by glucocorticoids *in vivo*[3,6], and by cyclic AMP both *in vivo*[6,7] and in cultured baby hamster kidney cells[8]. There have also been conflicting reports about the efficacy of polyamines in inhibiting the induction of ODC *in vivo*[9-11] and in activated human lymphocytes[12]. As most results reported so far are from whole animal studies, we felt that a more precise understanding of ODC induction

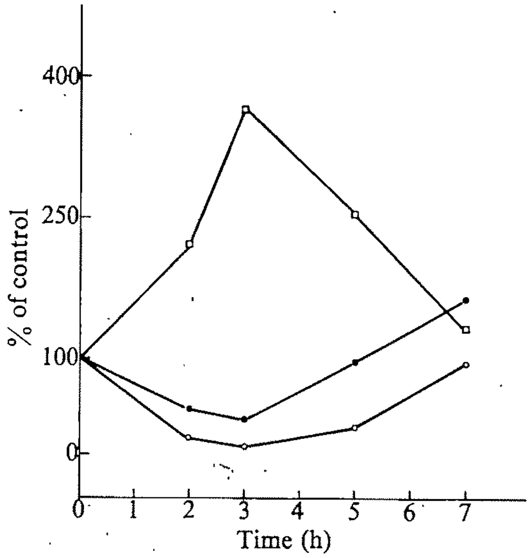

Fig. 1 Dibutyryl cyclic AMP induction of ornithine decarboxylase in intact HTC cells and the effect of 10^{-5} M spermine on non-induced and cyclic AMP-induced enzyme. Hepatoma tissue culture (HTC) cells (Morris rat Hepatoma 7288C) were provided by Dr Thomas Gelehrter and were maintained in tissue culture as described previously[14]. Twenty four hour logarithmically growing HTC cells (at a cell density of approximately 5×10^5 cells ml^{-1}) were collected by centrifugation at 1,700*g* for 10 min at 24 °C in a Servall. The cells were then suspended in induction medium (growth medium from which the sera have been deleted) to the same final concentration at which they were growing. All experiments were carried out in suspension culture at 37 °C in a G77 gyratory water bath shaker (New Brunswick Scientific). N^6, $O^{2'}$–dibutyryl adenosine 3′,5′-monophosphate (Boehringer-Mannheim) and dexamethasone-phosphate (Merck, Sharpe and Dohme) were dissolved immediately before use in the same Tris–EDTA buffer used in the ODC assay; spermine (ICN:K & K Labs) was also prepared in this manner. At each time point, aliquots of HTC cells containing 10^7 cells ml^{-1} were centrifuged at 1,700*g* for 10 min at 24 °C in a Servall. For routine assays 5×10^6 cells were used per assay and the assays were always done in duplicate. The cell pellet was resuspended in assay medium, the cells broken by sonication and debris was removed by centrifugation at 20,000*g* for 15 min at 4 °C. The supernatant was assayed for ODC activity as described before[15]. The assay buffer contained 50 mM Tris (*p*H 7.8 at 37 °C), 1 mM EDTA, 0.5 mM pyridoxal phosphate, 5 mM dithiothreitol and non-saturating levels of 6×10^{-6} M L-1–^{14}C-ornithine. □, Cyclic AMP (10^{-3} M); ○, spermine (10^{-5} M) or spermidine (6×10^{-5} M); ●, cyclic AMP (10^{-3} M)+spermine (10^{-5} M) or spermidine (6×10^{-6} M).

may result from investigation of an isolated system. The system chosen for detailed study was rat hepatoma cells (HTC) because most *in vivo* studies have focused on ODC from rat liver. We now report the induction of ODC in HTC cells by both cyclic AMP and dexamethasone.

Induction by cyclic AMP reached a maximum fourfold increase after about 3 h, and activity declined to basal levels by 7 h (Fig. 1). Induction by dexamethasone, on the other hand, reached a sixfold increase in activity by 12 h and remained at this level for more than 20 h in the presence of the inducing agent (Fig. 2). The difference between the two types of induction was investigated using spermine and inhibitors of RNA and protein synthesis. When spermine was added to the induction medium simultaneously with the inducing agent, 10^{-5} M spermine was sufficient not only to abolish the cyclic AMP induction, but also to depress ODC activity well below control level (Fig. 2). Spermidine at 6×10^{-5} M was equally effective as spermine. When ODC was induced by dexamethasone, neither spermidine nor spermine had any effect, even at concentrations of up to 10^{-2} M (Fig. 2).

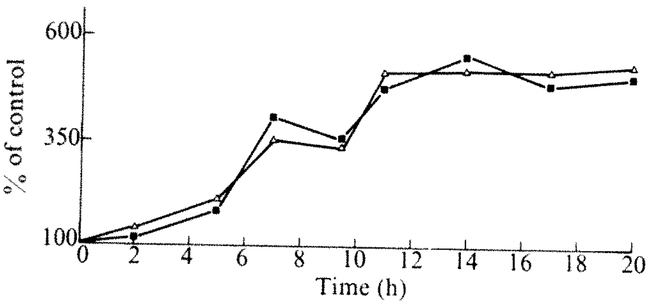

Fig. 2 Dexamethasone induction of ornithine decarboxylase in intact HTC cells and the effect of 10^{-5} M spermine on the induced enzyme. The conditions of the experiment are identical to those described for Fig. 1. △, Dexamethasone phosphate (2×10^{-6} M); ■, dexamethasone phosphate (2×10^{-6} M)+ spermine (10^{-5} M) or spermidine (6×10^{-5} M).

To time the inhibition by spermine of the induction of ODC, cyclic AMP and spermine were added simultaneously; spermine was then removed at different times by centrifuging the cells and resuspending them in induction medium containing that concentration of cyclic AMP present initially. In approximately 30 min (the time required for handling the samples), 10^{-5} M spermine completely inhibited induction by cyclic AMP. This rapid depression of ODC activity suggests a functional role for spermine in its control of ODC.

To investigate the relative effectiveness of spermine and inhibitors of RNA and protein synthesis with respect to induction of ODC, we added 10^{-5} M spermine or cycloheximide (25 μg ml^{-1}) or actinomycin D (5 μg ml^{-1}) at the same time as cyclic AMP or dexamethasone; ODC activity was then measured after 3 h for cyclic AMP and 15 h for dexamethasone. Table 1 shows that neither cycloheximide nor actinomycin D affected the basal enzyme level, whereas spermine inhibited it by 50%; furthermore, spermine was unique in affecting only cyclic AMP induction. Cycloheximide abolished induction by both agents; although actinomycin D completely inhibited induction by dexamethasone it only partially (65%) inhibited induction by cyclic AMP.

The low effective concentration of spermine, its speed of action, as well as its selectivity in inhibiting only the induction by cyclic AMP precludes feedback inhibition as the possible mechanism of its action. The cyclic GMP–cyclic AMP ratio and the spermine–spermidine ratio are considered to be important factors in regulation of ODC synthesis[15]. Results with cycloheximide verify the role of protein synthesis in induction of ODC. Continuous protein synthesis may not be necessary for basal ODC activity as this is not inhibited by cycloheximide; that this basal activity is affected by spermine suggests a unique role for this agent. The only partial inhibition by actinomycin D, which is observed also in cultured baby hamster kidney cells[8], indicates a post-transcriptional site of action for cyclic AMP in contrast to that of dexamethasone. The ability of ODC to fluctuate rapidly in response to the introduction of these agents suggests that elevation of ODC activity may be an important step in the control of cellular activity.

Table 1 Ornithine decarboxylase activity (p mol per mg protein per 30 min)

	Control	Cyclic AMP (10^{-3} M)	Dexamethasone (2×10^{-6} M)
Control	130±5	536±12	806±15
Spermine (10^{-5} M)	65±3	78±3	802±21
Cycloheximide (25 μg × ml^{-1})	130±7	130±2	128±3
Actinomycin D (5 μg × ml^{-1})	130±4	182±3	130±4

The effects of spermidine, cycloheximide, and actinomycin D on cyclic AMP-induced ornithine decarboxylase in whole HTC cells. The conditions of the experiment are those described for Fig. 1. Spermine, cycloheximide (Sigma) and actinomycin D (Merck, Sharpe and Dohme) were added at the time of addition of the inducing agent. The effects of these compounds were measured at 3 h for the cyclic AMP-induced ODC and at 15 h for the dexamethasone induced ODC enzyme.

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Received March 1; accepted May 8, 1975.

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Increased neural lobe content of neurophysins in rats treated with exogenous vasopressin

In a number of endocrine systems it has been established that negative feedback mechanisms are involved in the regulation of hormone synthesis and release. No such role has however, been established for vasopressin in the control of the hypothalamo-neurohypophysial neurosecretory system. The present experiments were carried out to determine the effects of administration of exogenous vasopressin on the stores of the neurohypophysial peptides oxytocin and vasopressin and the proteins (neurophysins) associated with them in the neurosecretory granules.

Groups of male Wistar rats (Porton strain) of approximately 200 g body weight were given daily intramuscular injections of either 0.5 IU vasopressin tannate in oil or the same volume (0.1 ml) of the vehicle alone. This dose of

Table 1 Mean (±s.e.) content of neurohypophysial vasopressin (pressor activity), oxytocin (milk ejection activity) and neurophysins A, B and C in rats injected with vasopressin tannate in oil (0.5 IU daily intramuscularly for 8 d) or arachis oil

Injection	Vasopressin ($\times 10^{-3}$ IU)	Oxytocin ($\times 10^{-3}$ IU)	Neurophysin (arbitrary units)		
			A	B	C
Oil	234.3±8.5	225.7±19.1	16.8±0.7	6.5±0.3	3.7±0.3
	NS	NS	$P<0.02$	$P<0.01$	$P<0.01$
Vasopressin	227.7±9.9	221.6±24.7	19.5±0.7	7.9±0.4	5.3±0.3

All values are presented in terms of metabolic size, that is, per (100 g initial body weight)$^{0.75}$ to take into account minor differences in body weight between animals[5]. The number of figures constituting each mean varied between 11 and 19. Results were compared using Student's *t* test.

NS, not significant.

vasopressin is sufficient to raise urinary osmolality and reduce water intake significantly without causing alteration of plasma osmotic pressure values[1]. After 8 d of treatment rats in the two experimental groups were killed and the neural lobes removed. Half of the glands from each group were homogenised in 0.05 M acetic acid and the extracts subsequently used for hormone bioassay. Vasopressin and oxytocin were assayed using respectively the rat vasopressor and rat milk ejection techniques. The other half of the glands were homogenised in a small volume (50 µl) of 0.1 M HCl and subjected to polyacrylamide gel electrophoresis as described previously[2]. After electrophoresis and a dye staining–destaining step the quantities of the three rat neurophysins (one minor and two major components) present in each gel were estimated using a densitometric method (C.W.J., unpublished) similar to that described previously[3].

The results (Table 1) indicate that in these conditions of vasopressin administration the neural lobe content of vasopressin and oxytocin was unaltered when compared with control animals. In contrast, however, there was a significant increase in the neural lobe content of all three rat neurophysins.

It is difficult to explain the accumulation of neurophysins, without any associated change in hormone content, in the vasopressin-treated animals. It may be that there has been an inhibition of the release of oxytocin, vasopressin and neurophysins from the neurohypophysis accompanied by a more rapid intracellular degradation of hormones than of neurophysins. A mechanism involving intracellular degradation has been postulated by Smith and Farquar[4], which removes excess hormone from adenohypophysial tissues. Although at present an element of speculation must remain in the interpretation of these findings, two points emerge clearly. First, either directly or indirectly, administration of high levels of exogenous vasopressin can influence the hypothalamo-neurohypophysial system. Second, the bioassayable hormone in the neurohypophysis may not always bear a constant relationship to the content of neurophysins.

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λ Lysogens of *E. coli* reproduce more rapidly than non-lysogens

THE evolutionary basis for the integration of phage DNA into bacterial chromosomes (lysogeny[1,2]) is not understood. A generally accepted explanation is that when the bacterial population is large and growing well, the phages prefer the lytic pathway since they can multiply without elimination of all host bacteria. If conditions deteriorate so that the supply of bacteria becomes limited or if growth is slowed, the phages tend to lysogenise. There have been few studies of the evolutionary mechanisms which may have selected for lysogeny. We now report that in certain growth conditions λ lysogens of *Escherichia coli* reproduce more rapidly than isogenic, non-lysogenic strains.

E. coli, AB 257 *met*, lysogenised with a temperature inducible phage λ (C_{I857}) grows normally at 30 °C but is lysed if the temperature is raised to 42 °C. Two other mutations in the phage prevent its induction or replication at 30 °C. The *ind* mutation[3] reduces the inducibility of the prophage to a very low level and the *sus*J mutation[4] makes phage production impossible in this non-permissive *E. coli* host. At 42 °C the prophage is induced by the thermolability of the C_I repressor protein and the host bacteria are lysed.

A mixed population of the lysogenic and non-lysogenic bacteria was grown in broth or M-9 medium[5]. Fig. 1 shows that there was little change in the frequency of the bacterial types in the population in these growth conditions. The ratio of the two bacterial types fluctuated from day to day but the frequency remained relatively contant in repeated experiments. The growth rates of the separate strains in M-9 medium indicated that both strains had a generation time of 80 min in either excess or limited glucose. Figure 1 shows that in broth there was no significant change in frequency after 80 generations.

If equal mixtures of the lysogenic and non-lysogenic bacteria were grown in a chemostat in which growth was limited by the glucose concentration of 0.01%, however, lysogenic bacteria rapidly replaced non-lysogenic bacteria (Fig. 1). The rate of increase of lysogenic bacteria varied in different experiments but the lysogen usually attained a frequency greater than 99% within 30 generations. At this concentration of glucose the population density was 2–3×10⁸ bacteria per ml and the flow of medium was adjusted to give 10–15 generations per day. In the chemostat

Table 1 Change in the frequency of the lysogenic *E. coli* in a mixed population after shift to a different growth medium

Shift	Generations	Lysogen frequency Before	Lysogen frequency After
Broth to broth	40	0.46	0.43
Broth to glucose	20	0.46	0.53
Glucose to broth	20	0.41	0.47
Exponential to stationary	40	0.46	0.43

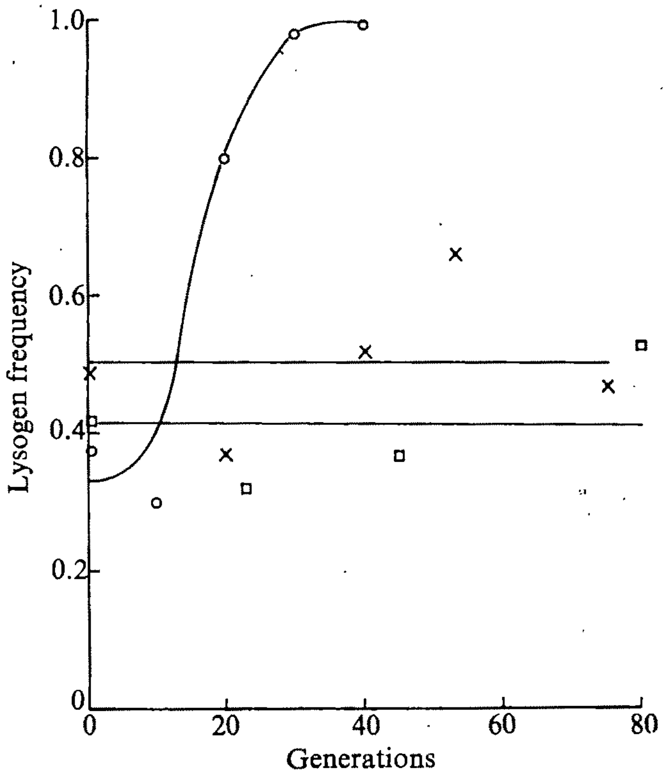

Fig. 1 Growth of a mixed population of lysogenic and non-lysogenic *E. coli* in different conditions. The lysogenic and non-lysogenic bacteria were grown separately overnight in M9 (0.2% glucose, 40 μg ml^{-1} methionine). A portion of each culture was mixed to give equal numbers of both types and the exact number of each type was determined by spreading a dilution of the mixed population on broth-agar plates and incubating duplicate plates at 30 °C and 42 °C. The number of colonies at 42 °C gives the number of non-lysogenic bacteria and the number of colonies at 30 °C gives the total number of bacteria. For the chemostat experiments the frequency of each type was measured in the initial inoculum, after overnight growth in the chemostat and at daily intervals thereafter. For the serial dilution experiments the mixed population was diluted 10^{6} daily into fresh medium and grown at 30 °C. The cultures were sampled daily. ○, Frequency of the lysogen in a chemostat; ×, frequency of the lysogen in M9 plus 0.4% glucose and 40 μg ml^{-1} methionine; □, frequency of the lysogen in broth.

experiment shown in Fig. 1, bacteria were grown separately overnight in 0.2% glucose and equal mixtures were diluted into a chemostat containing 0.01% glucose and grown overnight without additional medium. In other experiments each strain was grown separately overnight in 0.01% glucose and then a mixture was diluted into the chemostat. If the bacteria were adapted to 0.01% glucose, the population was converted to the lysogen more rapidly than is shown in Fig. 1.

Since the bacteria were being shifted from a medium of high glucose concentration (0.2%) to low concentration (0.01%), control experiments were done to determine the effect of abrupt changes in growth medium on the frequency of the strains in the population. Table 1 shows the results of nutritional shift-up and shift-down experiments. There is no significant change in the frequency of the two strains after shift from broth to M-9 or vice versa. In addition, the frequency of the two types was measured in exponential growth (10^{7} bacteria ml^{-1}), stationary phase (10^{9} bacteria ml^{-1}) and after continued incubation in stationary phase for more than 24 h. Table 1 shows that the initial and final frequency of the lysogen was unchanged throughout these changes in growth conditions.

To determine whether the lysogen strain was more fit than the non-lysogen strain in other conditions, mixed populations were grown in chemostats in which growth was limited by glycerol, lactose or acetate (all 0.01%) or b methionine (0.05 μg ml^{-1}). Figure 2 shows the change i limited glycerol or methionine. In limited glycerol the frequency of the lysogen in the population increased rapidly and the same increase was observed in limited lactose o acetate. Methionine limitation, however, did not significantly increase the frequency of the lysogen. In severa chemostat experiments in which growth was limited b methionine, the lysogen showed no persistent increase i frequency although the data in Fig. 2 show an initia increase and subsequent decline in frequency. The experiments with methionine limitation were complicated b selection for methionine, revertants in the chemostat and i was impossible to grow the population for long periods without reversion to prototrophy. The data suggest however, tha methionine limitation does not confer the same increase fitness that is evident when the carbon source is limited.

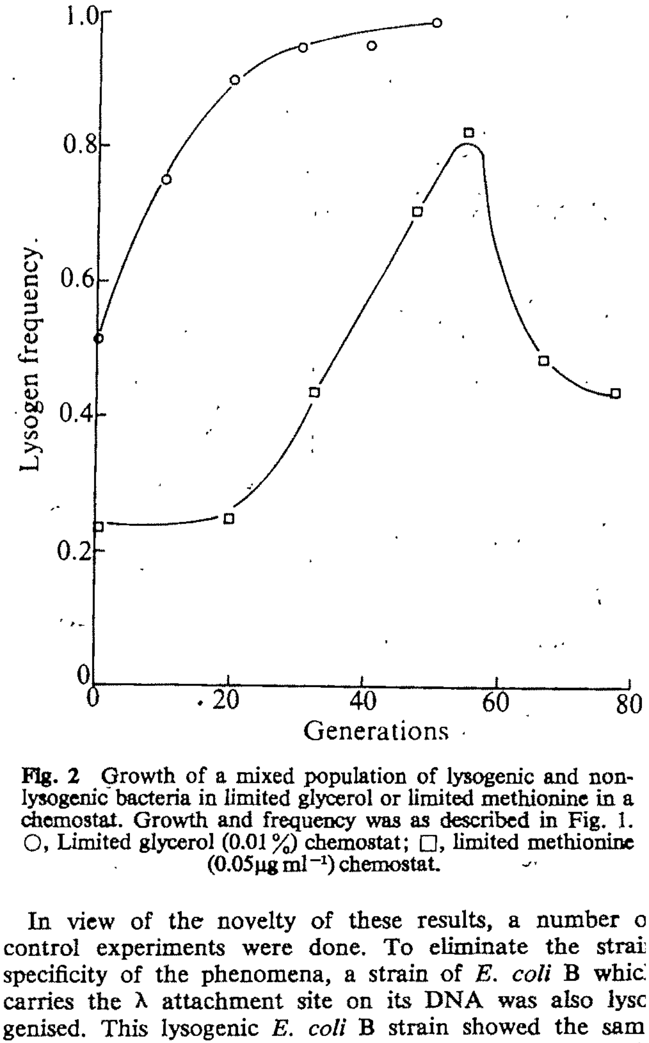

Fig. 2 Growth of a mixed population of lysogenic and non-lysogenic bacteria in limited glycerol or limited methionine in a chemostat. Growth and frequency was as described in Fig. 1. ○, Limited glycerol (0.01%) chemostat; □, limited methionine (0.05μg ml^{-1}) chemostat.

In view of the novelty of these results, a number o control experiments were done. To eliminate the strai specificity of the phenomena, a strain of *E. coli* B whic carries the λ attachment site on its DNA was also lysogenised. This lysogenic *E. coli* B strain showed the sam increased reproduction rate over the non-lysogenic strai when grown in a mixed population in limited glucose.

One unexplained aspect of the increased reproduction rat of the lysogen is the final frequency reached after extende growth in the chemostat. If a dilution of the lysogenic strai alone was spread on agar plates and incubated at 30 ° and 42 °C, fewer than 10^{-8} bacteria formed colonies a 42 °C. In all chemostat experiments performed the fina frequency of non-lysogenic bacteria was never less tha 10^{-4} and usually stabilised at 10^{-3}. This suggests tha although the lysogen is reproduced more rapidly than th non-lysogen in the chemostat, the non-lysogen is not eliminated from the population but is maintained at a lo frequency.

Some bacteria may attach to the walls of the chemosta thus maintaining the non-lysogenic bacteria in the culture. To test this, a sample from a chemostat in which the frequency of the two bacterial types had stabilised was diluted into an identical sterile chemostat and growth continued. No further change in frequency was observed, suggesting that attachment does not maintain the non-lysogen in the culture.

So far the data indicate that an *E. coli* bacterium carrying the prophage λ is more fit than a non-lysogen when growth is limited by the carbon source. In several other conditions this increased rate of reproduction is not conferred. We are testing the generality of this increased reproduction rate with regard to other prophages and chromosome attachment sites.

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Received March 24; accepted May 8, 1975.

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Reassembly of a spherical virus in mild conditions

ALTHOUGH successful reassembly of a number of spherical viruses has been reported[1–5], this has hitherto only been accomplished in conditions which are clearly non-physiological. We now report the reassembly of cowpea chlorotic mottle virus (CCMV) in mild conditions, ~*p*H 6, ionic strength 0.1 or 0.2 and 20 to 25 °C; conditions which are plausible for an *in vivo* assembly reaction.

The virus was grown as described previously[6] and the protein prepared by dissociation at high *p*H and ionic strength, followed by removal of the RNA either by calcium precipitation and ion exchange chromatography[6] or by sedimentation of the RNA[4]. RNA for reassembly was prepared by dissociation of the virus with 2% SDS at 50 °C and then either phenol extraction or sucrose gradient purification of the RNA. Reassembly was obtained either when the protein, weakly buffered to *p*H 8.0 at ionic strength 0.2, was mixed with a strongly buffered RNA solution (at *p*H 6.0, ionic strength 0.2) to give a final *p*H near to 6 at 25 °C, or alternatively when the protein and RNA were mixed at *p*H 7.5 and then dialysed rapidly to *p*H 6.0, ionic strength 0.1 at 20 °C. Neither of these procedures is necessarily the best possible choice, as the optimum conditions for the reassembly have not yet been sought, but even so with an RNA–protein ratio of 1 : 4 or 1 : 5 (compared with the proportion of 1 : 3.3 which occurs in the virus), at least 80% of the RNA becomes encapsidated within 2 h.

The resulting reassembled material has a sedimentation coefficient of 75–78S, compared with 77–79S for the normal virus: scans from sedimentation velocity experiments are shown in Fig. 1. Reassembled particles also have a similar molecular weight to native ones of about 4.5×10^6, as determined by sedimentation equilibrium, and a similar density in caesium chloride gradients. Electron micrographs of reassembled particles are again very similar to those of the original virus (Fig. 2) and besides the characteristic two-fold views marked in Fig. 2 (comparable with those shown for the capsid[7]) we have also seen three-fold, five-fold and local two-fold views which are similar to those found for the closely related broad bean mottle virus[8]. As a further check of the quality of the reassembled product, its infectivity was compared with that of native CCMV in a dilution assay on cowpea plants. Both produced systemic infections when inoculated at concentrations of 0.1 mg ml^{-1} but not at 0.02 mg ml^{-1}, while the RNA used for the reassembly did not produce infection at 0.02 mg ml^{-1} (corresponding to 0.09 mg ml^{-1} virus). Although this is only a preliminary comparison of the infectivities, this distinction between the native and reassembled CCMV on the one hand and their isolated RNA on the other is again compatible with the resemblance of our reassembled material to native undissociated CCMV.

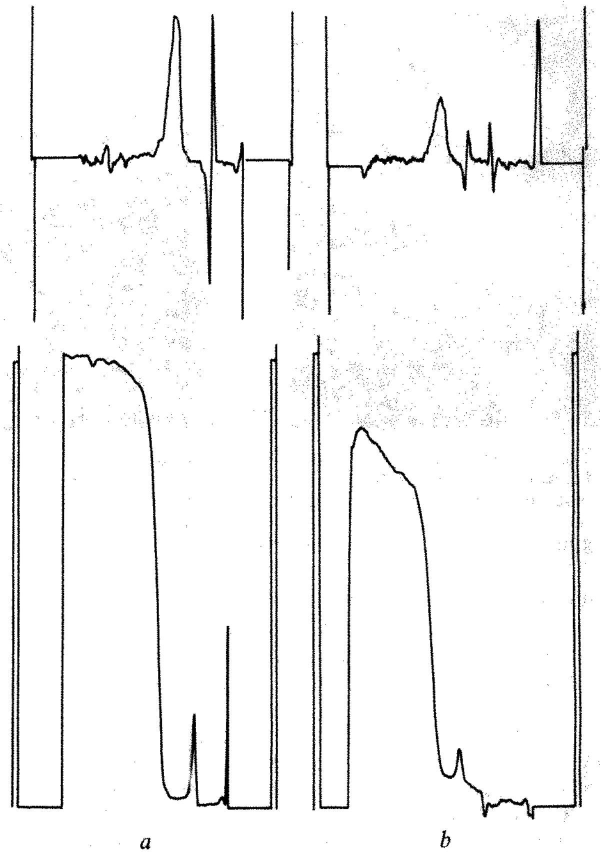

Fig. 1 Sedimentation of native (*a*) and reassembled (*b*) CCMV observed in the analytical ultracentrifuge. Samples in sodium phosphate, *p*H 6.0, *I* 0.2 at 20 °C centrifuged at 20,000 r.p.m. Scans at 260 nm were taken approximately 15 min after reaching full speed. Lower curves show the absorbance along each cell, with the derivative curve above.

In the final conditions used for this reassembly, a solution of the protein alone would, at equilibrium, contain less than 10% capsid (with sedimentation coefficient around 50S) and over 90% small aggregates (around 3S)[6], but there is a marked hysteresis on going from the disaggregated state (for example, *p*H 7.5, ionic strength 0.2) to the capsid[6]. This hysteresis was observed over a period of hours at both 5 and 23 °C, but equilibrium appears to be established within 48 h. Also in these conditions, an hexagonal array of CCMV protein subunits is frequently observed on the specimen grids used for electron microscopy, and we had speculated[6] that this might be due to a nucleation barrier for the protein aggregation which is overcome on interaction with the carbon surface of the grid and that it may also be overcome by interaction with the viral RNA. But direct mixing of the equilibrium protein and RNA did not lead to the reassembly of stable virus-like particles, but only to the formation of more capsid. To obtain such reassembly, it was necessary to supply the protein in a non-equilibrium form, by taking advantage of the hysteresis as already described. The difference in the properties of equilibrium and non-

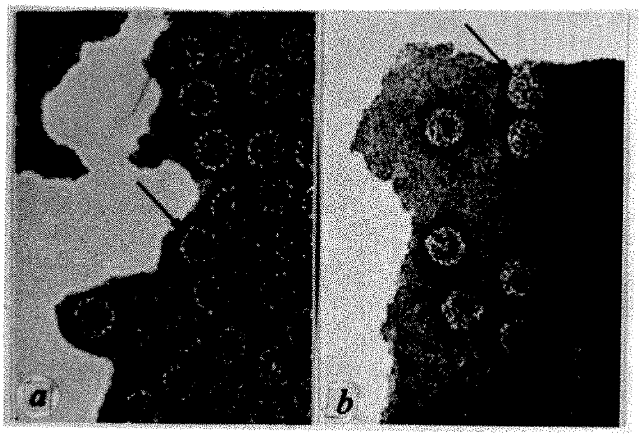

Fig. 2 Electron micrographs of (*a*) native and (*b*) reassembled CCMV. Marked particles show the twofold view characteristic of particles with this surface morphology. Samples were negatively stained with 1% (w/v) uranyl acetate and photographed over holes in the carbon substrate. (×225,000).

equilibrium protein at *p*H 6 is presumably due to the greater degree of aggregation of the former, while the less aggregated non-equilibrium material may be in a state closer to that of freshly synthesised coat protein in a natural infection.

The reassembly we describe is highly reproducible and occurs to a substantial extent over the range from *p*H 5.5 to 6.5, ionic strength 0.1 to 0.5 and temperature between 5 and 25 °C. In addition, we have not, so far, found any marked effect of magnesium or polyamine ions, in contrast to their requirement for satisfactory reassembly of CCMV from high ionic strength[5]. We have therefore found reassembly within the region of physiologically plausible conditions, although these can clearly be refined by further work. Thus, in spite of its close similarity to the native virus, our reassembled product is less stable in caesium chloride and we think that this may be due to the lack of some annealing process during or after the main assembly. The way is now open for further investigation both of this process and of the selectivity of the virus protein for its multiple RNA species.

Previously, the reassembly of spherical viruses has required the presence, and subsequent removal, of denaturing agents (such as 5.7 M urea for R17 (ref. 1) or 8 M guanidine hydrochloride for Qβ (ref. 2)) or of high concentrations of salt at low temperature (for cucumber mosaic virus[3] and the CCMV group[4,5]). Clearly such reassembly departs, at least in this respect, from the normal assembly of the virus and may not even follow the same pathway. We believe that the more significant processes will be found to occur near neutrality, at ionic strengths of 0.1 to 0.2 and temperatures from 20 to 37 °C. In particular, the phenomenon of selectivity of the protein for its homologous RNA may well be best observed in such conditions, as in the case of the rod-shaped tobacco mosaic virus[10,11]. Another similarity with the reassembly of tobacco mosaic virus lies in the requirement for the protein to be in the correct aggregation state for the optimum reaction, that is, the disk for tobacco mosaic virus[11] and the "hysteresis protein" for CCMV.

We thank Dr B. Jacrot of the Institut Max Von Laue-Paul Langevin, Grenoble, France, for helpful discussion. K.W.A. was supported by a postdoctoral fellowship from the American Cancer Society.

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Received December 13, 1974; accepted May 16, 1975.

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A complex genetic locus that controls the first three steps of pyrimidine biosynthesis in *Drosophila*

IN eukaryotes, the initial steps in the *de novo* synthesis of pyrimidines are performed by multienzyme complexes consisting of the first two enzymes in fungi and the first three enzymes in mammals[1,2]. In *Neurospora* there is evidence that this complex facilitates channelling of the carbamyl phosphate produced by pyrimidine-specific carbamyl phosphate synthetase (CPSase) to the second enzyme, aspartate transcarbamylase (ATCase)[3]. The aggregation of these enzymes in fungi correlates with close linkage of the structural genes for both CPSase and ATCase which are apparently cotranscribed and translated[4–7]. In fungi the third pyrimidine pathway enzyme, dihydroorotase (DHOase) is encoded by an unlinked gene[5,8]. Considering the close relationship between enzyme aggregation and gene clustering in this system in fungi, the presence of the first three steps of pyrimidine biosynthesis in a single mammalian enzyme aggregate led us to question whether all three structural genes may be clustered in animals. We now report that this is the case in *Drosophila* and that the fourth enzyme in the pathway is not included within this gene cluster.

The sex-linked, recessive mutation *rudimentary* (*r*) wing affects wing morphology and female fertility in *D. melanogaster*[9,10]. Genetic complementation occurs among certain *r* alleles[11] and complementation maps of the locus are colinear with fine structure maps[12,13]. Carlson has defined seven complementation units, I–VII (ref. 13) (Fig. 1). Subsequent to these detailed genetic studies, *r* flies have been shown to be auxotrophic for pyrimidines or pyrimidine precursors[14,15]. Based on nutritional studies and direct assay of ATCase, Nørby proposed that the *r* locus controls at least the first two steps in *de novo* pyrimidine biosynthesis, CPSase and ATCase[16,17]. Specifically, he showed that complementation unit I alleles lack ATCase and proposed that unit VI alleles lack CPSase. This hypothesis has been verified by Jarry and Falk and extended to include unit III within the ATCase zone of the *r* locus[18]. These workers also found normal levels of both CPSase and ATCase in flies possessing a complementation unit VII allele. From our own work we find that flies of this complementation class are deficient in activity of DHOase. This observation coincides with one of the suggestions for the function of this complementation unit independently put forward by Jarry and Falk[18].

Table 1 Complementation among *r* alleles

		I–VII (r^{60d1})	VII (r^{3710})	VI (r^{39k})	I–IV (r^{59k23})
I–IV	(r^{59k23})	−	+	+	−
VI	(r^{39k})	−	+	−	
VII	(r^{3719})	−	−		
I–VII	(r^{60d1})	−			

Alleles were combined in *trans* heterozygotes which were scored for wing phenotype. Complementation group designations are in Roman numerals while the actual allele used is noted in parentheses. These mutants are described in detail elsewhere[13]. Complementing combinations (+) yield normal wings while non-complementing combinations (−) yield *rudimentary* wings.

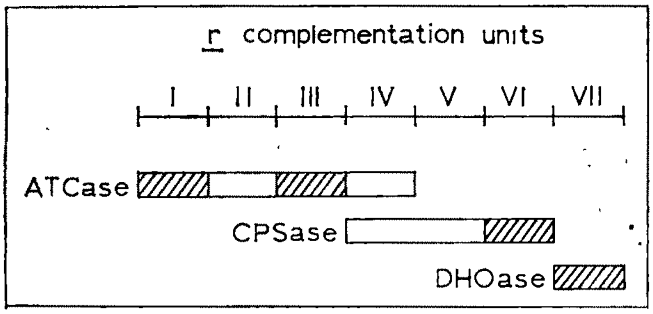

Fig. 1 Enzyme-specifying regions of the *rudimentary* complementation map. Complementation unit designations are from Carlson[13]. Horizontal bars indicate those units for which control of ATCase, CPSase, or DHOase levels is demonstrated (cross-hatched) or suspected (open). See text for discussion.

Allele complementation with respect to the wing phene was verified for the *r* alleles used in this study. Table 1 shows that for alleles representing complementation groups I–IV, VI and VII, all possible heteroallelic combinations yielded normal winged flies, whereas the group I-VII allele failed to complement any other allele.

Previous studies have associated *r* complementation groups I–VII, V–VI and VI with CPSase deficiencies and groups I–VII, I and III with ATCase deficiencies[16,18]. Table 2 corroborates the latter findings, showing ATCase activity to be greatly reduced by complementation group I–IV and group I–VII alleles. This enzymatic lesion is not found with alleles of group VI or group VII.

The third enzyme of the pyrimidine pathway, DHOase, is specified by complementation unit VII of the *r* locus. Table 2 shows that while flies homozygous for group I–VII or group VII alleles possessed much less DHOase than normal, activity was essentially normal in group I–IV and group VI individuals.

A map of the enzyme functions of the *r* locus is presented in Fig. 1. Complementation units I and III specify ATCase activity[16,18]; unit VI directs CPSase activity[19]; unit VII is responsible for DHOase levels (Table 2). It is unlikely that any of the three remaining *r* complementation units individually specify an unknown *r* function since their existence is only inferred and no *r* allele uniquely belongs to any of these units[13]. Unit II is presumably involved in ATCase expression since it lies between units I and III. Unit IV could affect ATCase and or CPSase levels. Unit V probably directs CPSase along with unit VI since an allele belonging to complementation group V–VI has normal ATCase and lacks CPSase[18]. It must be pointed out that group IV–V alleles are known[13]; thus it is conceivable that these two units are jointly responsible for an unknown function of the *r* locus.

If an additional enzymatic function is specified by *r*, apparent biochemical and genetic correlations suggest later steps in the pyrimidine pathway. Accordingly, the fourth enzyme, dihydroorotate dehydrogenase (DHOdehase), was assayed in flies homozygous for a totally non-complementing allele. Table 3 shows that if anything, these flies had more DHOdehase activity than wild type flies. Thus, the ability to convert dihydroorotate to orotate is not diminished in *r* individuals.

It is important to know whether the *r* locus encodes the primary structures of the early pyrimidine enzymes or if it is a regulatory site, the product of which is essential for the synthesis, stability, or activation of these enzymes. Suggestive evidence regarding these possibilities is gleaned from the response of enzyme activity to changes in gene dose, thus Table 2 also presents enzyme values for flies heterozygous for *r* mutations. Clearly, alleles of *r* are codominant with respect to ATCase and DHOase activity; that is, females possessing one mutant and one wild type dose of the locus have enzyme levels intermediate between mutant and wild type homozygotes. Flies that possess three normal doses of the ATCase and DHOase regions of *r* ($r^{39k}/r^{39k}/Dp(1;4)r^{+}f^{+}$) have approximately 50% more ATCase and DHOase than flies with two doses of these regions, which suggests that the availability of *r* gene copies is rate-limiting in the expression of these enzymes. Since strict proportionality between gene dose and enzyme activity might be an exclusive property of enzyme structural genes[22,23], we

Table 2 ATCase and DHOase activities in females bearing various *r* alleles

Complementation group	Genotype	ATCase	DHOase	*n*
Wild type homozygote	r^{+}/r^{+}	76 ± 15	26 ± 4	4
I-IV Homozygote	$g^{2}\, r^{59k23} f / g^{2}\, r^{59k23} f$	4 ± 1	17 ± 4	3
Heterozygote	$g^{2}\, r^{59k23} f / Ins(1)\, sc^{S1}+d1-49,\, v\, r^{+}$	42 ± 8	17 ± 1	3
VI Homozygote	$g^{2}\, r^{39k} f / g^{2}\, r^{39k} f$	83 ± 25	21 ± 6	3
Heterozygote (aneuploid)	$g^{2}\, r^{39k} f / g^{2}\, r^{39k} f / Dp(1;4)\, r^{+} f^{+}$	131 ± 12	33 ± 2	2
VII Homozygote	r^{3719} / r^{3719}	89 ± 21	4 ± 2	5
Heterozygote	$r^{3719} / In(1)\, ClB,\, v\, r^{+} B$	92 ± 23	14 ± 2	5
I-VII Homozygote	$r^{60d1} f / r^{60d1} f$	6 ± 1	4 ± 1	4
Heterozygote	$r^{60d1} f / In(1)\, FM6,\, y\, w\, dm\, r^{+}$	41 ± 6	12 ± 2	4

Complementation group designations are after Carlson[13] and all genetic markers and chromosomal rearrangements are described in detail elsewhere[13,10]. Wild type females are from an Oregon-R strain. Flies were cultured at 25 °C on standard cornmeal–molasses–agar medium that was supplemented with 1% RNA to promote viability of *r* genotypes. Adult females 2–4 d of age were frozen at −20 °C for 1–4 d before assay. Samples were homogenised with glass tissue grinders at a concentration of 250 mg flies per ml of cold 50 mM potassium phosphate buffer (*p*H 7.2), 250 mM in sucrose. Homogenates were cleared by centrifugation for one hour at 20,000*g* and the supernatant fractions were desalted on small Bio-Gel P-2 columns equilibrated with buffer less sucrose. ATCase activity was determined by incubating a 500 µl mixture containing 200 mM Tris-HCl (*p*H 9.2), 20 mM potassium L-aspartate, 15 mM repurified dilithium carbamyl phosphate[19], and 200 µl desalted extract. DHOase activity was measured in a 500 µl mixture containing 200 mM Tris-HCl (*p*H 8.0), 2 mM 4,5-dihydro-L-orotate, and 300 µl desalted extract. Mixtures were incubated one hour at 30 °C and the carbamylaspartate formed was determined by the method of Gerhart and Pardee[19]. Values are derived from the difference between samples and blank reaction mixtures from which substrate was omitted. Activities are expressed as mean nmol carbamylaspartate formed per h per mg protein ± s.e. Protein was determined by the method of Lowry *et al.*[20]. *n* is the number of separate samples assayed.

Table 3 DHOdehase levels in wild-type and *r* females

Genotype	DHOdehase activity
r^+/r^+ (Wild type)	16.0 ± 1.3
$r^{60\,d1} f/r^{60\,d1} f$ (Complementation group I–VII)	24.6 ± 0.8

Females from an Oregon-R wild type strain and from a homozygous strain of $r^{60\,d1}$ were reared and homogenised as described in Table 2 except that they were not frozen before homogenisation Homogenates were cleared of large cuticular debris by filtration through several layers of cotton gauze and glasswool. DHOdehase activity was estimated by incubating 10 µl filtered homogenate in a total volume of 50 µl including 200 mM Tris-HCl (*p*H 8.0) and 2 mM 4,5-dihydro-DL-orotate (6-^{14}C, New England Nuclear) (2.8 µCi µmol^{-1}). This mixture was incubated at 30 °C for 40 min. The reaction was stopped by adding 10 µl 10 N formic acid and the samples were frozen until chromatography. After centrifugation at 10,000*g* for 15 min to remove denatured protein, 5 µl sample and 5 µl of a carrier mixture (0.125 µmol each of N-carbamyl-DL-aspartate, 4,5-dihydro-DL-orotate, and L-orotate) were mixed and spotted on to sheets of DEAE-cellulose paper (Whatman, DE-81). The sheets were developed for 3 h at room temperature using a formic acid–methanol–water solvent system[21] With this method orotic acid ($R_f = 0.45$) is cleanly separated from dihydroorotate ($R_f = 0.70$). Carbamylaspartate, which is generated by endogenous DHOase activity, chromatographs at $R_f = 0.80$. ^{14}C-orotate is not further converted to orotidylate or uridylate because of lack of phosphoribosylpyrophosphate in these reaction mixtures. The orotic acid zone of each chromatogram was located by its flourescence under ultraviolet light, cut out, and ^{14}C-orotate was quantitated in a liquid scintillation system. Activity is expressed as mean nmol orotate formed per 40 min per mg protein ± s.e. Each mean is derived from determinations on three different samples.

Protein was determined by the method of Lowry *et al.*[20].

believe that the *r* locus contains the structural genes of the first three enzymes in pyrimidine biosynthesis.

In addition to providing genetic insight into the early steps of the pyrimidine pathway in animals, the presence of multiple cistrons within this locus in *Drosophila* has several implications for gene regulation and genome structure of higher eukaryotes. Most non-complementing *r* alleles are clustered near the ATCase end of the fine structure map[12,13]. Such polar mutants could define a *cis*-dominant regulatory element or may represent frameshift and nonsense mutations within a cotranscribed and translated multicistronic region. Thus, polycistronic mRNA could mediate the synthesis of the early pyrimidine enzymes in animals as well as in fungi[4–7]. Secondly the estimated size of the *r* locus, 24,000 base pairs of DNA, agrees closely with the average DNA content of a *Drosophila* chromomere, suggesting that this multicistronic locus resides within a single chromomere[24]. If so, *rudimentary*, and other "complex" loci are exceptions to a "one gene-one band" hypothesis[25], and it becomes imperative to determine the precise cytogenetic location of *r*. Lastly, a variety of genetic tests (frequency of gene conversion, intralocus map distance, complementation) indicate that the *r* locus is but one of a group of "complex" loci (for example, *white, Notch, dumpy, lozenge* and *miniature*)[24] that have a multicistronic nature.

We acknowledge helpful discussions with M. M. Green, comments on the manuscript by J. L. Ingraham and P. St Lawrence, and technical assistance from S Kuniyuki We thank D. Falk and B Jarry for access to their manuscript before publication This investigation was sponsored by a research grant, a training grant, and a postdoctoral fellowship from the National Institute of General Medical Sciences.

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Received January 20; accepted May 14, 1975.

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Radiation-induced DNA strand breaks in *E. coli* measured within a fraction of a second

THE yield of radiation-induced strand breaks in DNA is in normal conditions, larger in oxygenated cells than in anoxic cells. This may reflect either radiochemical reactions or preferential repair of breaks formed in anoxia. It is possible to distinguish between these possibilities by experiments in which irradiation and cell lysis are completed within a time period sufficiently short to minimise repair. We have measured in oxic and anoxic cells the yield of strand breakage on bacteriophage λ DNA superinfecting lysogenic bacteria[1] and now on chromosomal DNA in experiments where irradiation and lysis are carried out within a fraction of a second.

Figure 1 shows the results obtained when bacteria labelled with ^{3}H-thymidine were irradiated, rapidly lysed and then sedimented in alkaline sucrose gradients with non-irradiated ^{32}P λ DNA as a marker. The broad distribution of chromosomal DNA is as expected for a polydisperse collection of DNA molecules resulting from random strand breakage. After a dose of 12–13 krad in oxygen the peak of the ^{3}H-DNA sediments to the same position as does the ^{32}P λ marker DNA, the molecular weight of which is 15×10^6. For randomly broken DNA, the peak of radioactivity in an alkaline sucrose gradient occurs at a molecular weight that is 1.62 times greater than the average molecular weight[2]. Thus, the average molecular weight of the chromosomal DNA after a dose of 12–13 krad is 9.3×10^6. From these values, the yield of breaks in chromosomal DNA is calculated to be 8.3 to 8.9×10^{-12} breaks per dalton per rad. In nitrogen anoxia, about 35 krad are needed to make the peak of the ^{3}H-DNA cosediment with the ^{32}P λ marker DNA. Thus, in nitrogen the yield of breaks in chromosomal DNA decreases about threefold to 3.0×10^{-12} breaks per dalton per rad.

An increase in strand breaks in anoxic bacteria was found in *Micrococcus radiodurans* irradiated at high *p*H in the presence of EDTA (ref. 3) and in *Escherichia coli* subjected to heat treatment, osmotic cold shock, N-ethylmaleimide or quinacrine[4]. The authors[3,4] assumed that these treatments inhibited strand joining and concluded that the difference in radiation-induced strand breakage observed in oxygenated and anoxic cells is not caused by a difference in the initial number of radiochemical strand breaks, but by a novel and rapid repair mechanism that preferentially rejoins breaks produced in anoxia. If the oxygen effect observed for strand breakage in chromosomal DNA (Fig. 1) is caused by preferential repair, the repair process must have gone to completion within less than 200 ms in buffer at room temperature. From recent experiments[5], some conclusions can be drawn about the probable extent of strand joining in the conditions used in these experiments. The authors[5] estimated that the maximum number of single-strand breaks in an *E. coli* cell at 30 °C that could be rejoined per second is about 130. At 20 °C the activity of *E. coli* DNA ligase is only 0.57 of that

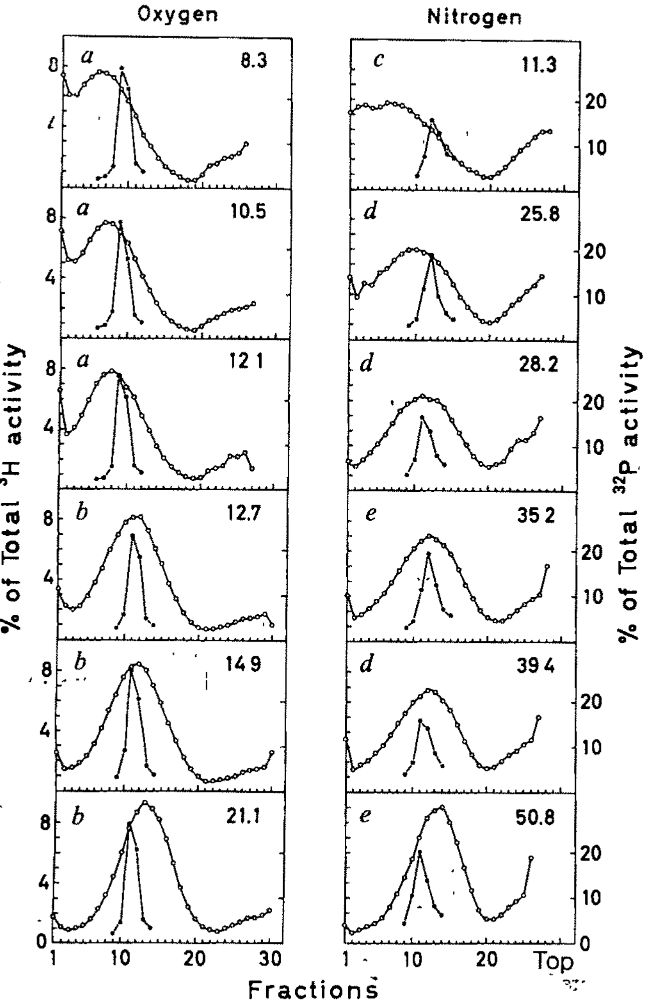

Fig. 1 Sedimentation profiles of chromosomal DNA after irradiation and lysis within a fraction of a second. Suspensions of *E. coli* K12 strain AB1886, ^{3}H-labelled in the DNA, in phosphate buffer (*p*H 6.8, room temperature) were equilibrated with oxygen or high purity nitrogen (<1 p.p.m. O_2). The cell suspensions were exposed to 4 MeV electrons from a linear accelerator with the doses indicated (krad), and rapidly transferred to alkaline detergent[1]. Times required to complete the experiments were between 75 and 95 ms for the oxygenated series and between 70 and 330 ms for the anoxic series, and include 45 ms needed for transfer of the cells to the detergent. The lysates were sedimented in 5–20% alkaline sucrose gradients together with non-irradiated ^{32}P-labelled phage λ DNA (Beckman SW50.1 rotor, 40,000 r.p.m., 20 °C, 2 h). Ten drop fractions were collected from the bottom of the tubes directly into scintillation liquid and the ^{3}H (○) and ^{32}P (●) activities measured for each fraction. Identical letters indicate that the profiles were obtained from the same run.

at 30 °C (ref. 6). Thus, at room temperature the maximum estimated number of strand-joining events would be less than 15 per cell in 200 ms. This can be compared with the number of strand breaks present in the cell after exposure to 4 MeV electrons. For an experiment of 200 ms duration about 27 krad are delivered. Assuming that in our growth conditions there is 15×10^{-16} g DNA per cell[7], there will after 27 krad be about 700 breaks per anoxic cell, and about 2,100 breaks per oxic cell. Thus, if rapid strand joining is to account for the difference of 1,400 breaks in oxic and anoxic cells, the strand-joining capacity must be at least 90 times greater than that estimated by Modrich and Lehman[6].

In separate experiments, the time needed for joining λ DNA molecules broken by pulsed electron radiation was measured[8]. In growth conditions, no strand joining was observed during the first 3 s of incubation. In the presence of oxygen most strand breaks were rejoined during 40 s incubation, whereas the rejoining was even slower in nitrogen anoxia.

Table 2 Effect of high *p*H on cellular sulphydryls

Treatment*	Sulphydryls in cell free supernatant (mM)
Phosphate buffer, *p*H 6.8	0.05
Veronal buffer, *p*H 8.6	0.57
Veronal buffer, *p*H 8.6+0.02 M EDTA	0.59
Tris buffer, *p*H 8.6	1.22
Tris buffer, *p*H 8.6+0.02 M EDTA	0.91

*Cells of *E. coli* K12 from a 100 ml log-phase suspension were collected by centrifugation and washed in phosphate buffer at *p*H 6.8. The cells were resuspended in 0.3 ml of the buffer as indicated, kept for 10 min at room temperature and the concentration of sulphydryls measured in the supernatant.

As we have found no experimental evidence for the existence of a very rapid strand break repair mechanism in anoxic cells, we do not believe that the explanation of the oxygen effect on observed strand breaks involves repair. The possibility that the experiments demonstrating changes in the oxygen effect by chemical and physical treatment of cells before irradiation[3,4] may reflect changes in the concentration of sulphydryl compounds in the cell, was tested in separate experiments. Table 1 shows that after a heat treatment of 52 °C for 10 min[4] more than 50% of the acid-soluble sulphydryls in the cells have leaked out into the suspending medium. This is paralleled by an increase in the number of radiation-induced DNA strand breaks in hypoxic and anoxic conditions. Thus, heat-shocked cells are sensitised twofold by a small contaminating concentration of oxygen (125 p.p.m.). The same effect is observed in cells treated with the sulphydryl-binding agent N-ethylmaleimide[9]. A slightly milder heat treatment, 48 °C for 5 min, had neither significant effect on the leakage of sulphydryls out from the cells nor on the yields of radiation-induced DNA strand

Table 1 Effect of heat shock on cellular sulphydryls and on DNA strand breakage

Treatment*	Sulphydryls in cell free supernatant (mM)	1/*e* dose for DNA single-strand breaks† (krad)		
		O_2	0.0125% O_2	N_2
20 °C, 10 min	0.19	7.1	—	38.7
Heat shock: 52 °C, 10 min	0.74	5.0	18.2	12.5–35.0‡
Heat shock: 48 °C, 5 min	0.24	6.1	34.7	40.5

*Cells of *E. coli* K12 from a 100 ml log-phase suspension were collected by centrifugation, suspended in 0.3 ml phosphate buffer at *p*H 6.8 and subjected to temperatures as indicated. After treatment the concentration of sulphydryls was measured in the cell free supernatant by the sodium nitroprusside reaction[11]. When the bacteria were suspended in 0.3 ml TCA at 0.3 M the concentration of sulphydryls in the supernatant was 1.38 mM.

†X-ray induced DNA single-strand breaks were measured in superinfecting, covalently closed circular phage λ DNA by sedimentation in alkaline sucrose[1].

‡X irradiation caused an exponential decrease in the number of intact circular DNA molecules, but the 1/*e* dose observed in individual experiments varied between the values shown. In the presence of $Na_2S_2O_4$ at 500 μM—a substance that combines quickly with oxygen—a 1/*e* dose of about 33 krad was repeatedly found.

breaks. Table 2 shows that a substantial amount of acid-soluble sulphydryls leaks out of the cells when suspended in veronal buffer at *p*H 8.6 supplemented with EDTA at 0.02 M (ref. 3). This effect is not caused by the presence of EDTA as it is observed with veronal buffer alone. The effect is more likely a result of the high *p*H as a substantial leakage of sulphydryls is also observed from cells suspended in Tris buffer at *p*H 8.6.

On this basis, we think that previous experiments[3,4] showing diminished anoxic protection for single-strand breaks in the treated cells, have been misinterpreted and should not be attributed to the loss of a rapid enzymatic repair process. Instead, we suggest that the effect may be caused by the fall in the level of sulphydryl compounds, which are known to act as hydrogen donors in radiochemical reactions and to contribute to anoxic radiobiological protection[10].

We thank Mr A. Rønnestad for assistance in operating the Linac at Norsk Hydro's Institute for Cancer Research. This work was supported by the Norwegian Council for Science and Humanities and the Norwegian Cancer Society.

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Received December 31, 1974, accepted May 14, 1975.

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Acquisition of tumour-inducing ability by non-oncogenic agrobacteria as a result of plasmid transfer

IT has been suggested that the tumour-inducing capacity of *Agrobacterium* is determined by plasmid genes[1,2]. The acquisition of virulence by non-pathogenic agrobacteria from pathogenic agrobacteria has been demonstrated by Kerr[3,4]. We investigated whether this phenomenon could be the result of transfer of a plasmid. Besides providing further evidence for the hypothesis that plasmid genes carry the genetic information for the tumour-inducing ability of *Agrobacterium*, the system offers the possibility of studying the genetics and functions of the plasmid.

The donor and recipient strains of *Agrobacterium* must be well-characterised. Donor strain *A. rhizogenes* 223, used in a first set of experiments, has the typical biochemical properties of cluster 2 (ref. 5). It cannot produce 3-ketolactose, does not grow anaerobically on nitrate containing medium, grows slowly and is resistant to all of the seven different types of virulent phages of our typing set (see Table 1). The cured non-tumour-inducing receptor strain C58-C1 was derived from a single colony of *A. tumefaciens* C58 by growth at 37 °C, which resulted in both the loss of its plasmid and the ability to induce tumours[2]. From C58-C1 a double mutant was isolated which showed resistance to 100 µg ml^{-1} rifampicin and to 300 µg ml^{-1} streptomycin. This C58-C1 *rif str* was repeatedly tested for plasmid and tumour-inducing ability and was always found to be negative for both. *A. tumefaciens* C58-C1 *rif str* belongs to the TT9 group of cluster 1 (ref. 5), can produce 3-ketolactose, can grow anaerobically and shows, among our laboratory strains, a unique and typical sensitivity pattern to the virulent phages of our typing set (see Table 1). We crossed the oncogenic *A. rhizogenes* strain 223 with the non-oncogenic *A. tumefaciens* strain C58-C1 *rif str* on *Kalanchoë daigremontiana* as host plant. The experimental procedures were as described by Kerr[4]. Eight weeks after inoculation 15 out of 30 reisolated single colonies of the antibiotic-resistant recipient bacteria seemed to be oncogenic on 1-week-old sunflower seedlings. A pathogenic colony was then subjected to further extensive purification. The resulting bacteria showed the antibiotic resistance, positive 3-ketolactose test, anaerobic growth and the phage sensitivity pattern characteristic of the avirulent C58-C1 *ref str* strain used as recipient (Table 1). They were repeatedly found to induce crown gall tumours on pea seedlings, sunflower seedlings and *Kalanchoë daigremontiana.* Using ultracentrifugation[1,2] and subsequent electron microscopy we demonstrated, in the purified newly oncogenic recipient bacterium, a large plasmid with a mean length of 58.0 µm±1.3 µm s.d., which lies in the range 61.1 µm±1.4 µm s.d. that we found for a sample of plasmid molecules isolated from the donor strain 223. *A. tumefaciens* C58-C1 *rif str* also acquired other characteristics that are known to be coded for by the plasmid, namely sensitivity to agrocins 84 and 396 (G. Engler, J.P.H., M. Holsters, M.V.M., R.A.S., and J.S., unpublished), the ability to degrade an arginine derivative nopaline (ref. 6 and G. M. Bomhoff, R.A.S., J.P.H., H. Kester, and J.S., unpublished) and the ability to exclude the virulent *Agrobacterium* phage AP1 (J.P.H., M.V.M., and J.S., unpublished). Strain C58-C1 *rif str* (TI223) differs from the parental oncogenic strain C58 in that it cannot be cured of its plasmid by growth at 37 °C, even after 75 generations, whereas a culture of the parental strain C58 is cured completely after 25 generations of growth at 37 °C. This property apparently depends on the nature of the plasmid present in the bacterium.

To confirm these results we performed a better controlled experiment on otherwise sterile pea seedlings in sterile test tubes containing agar medium. This cross involved the crown gall-inducing *Agrobacterium tumefaciens* strain Kerr 14 and the naturally occurring non-oncogenic *Agrobacterium radiobacter* S1005, made resistant against 100 µg ml^{-1} rifampicin and 300 µg ml^{-1} streptomycin. The pea seedlings were inoculated with S1005 *rif str* 12 d after inoculation with *A. tumefaciens* Kerr 14. As controls we used pea seedlings that were only inoculated with either Kerr 14 or S1005 *rif str.* Using sensitivity to agrocin 84 as a screening test we were able to pick up a single colony that was still resistant to 100 µg ml^{-1} rifampicin and to 300 µg ml^{-1} streptomycin, had the typical sensitivity pattern of *A. radiobacter* S1005 in a test with our typing set of seven different virulent *Agrobacterium* bacteriophages (Table 1), still produced the typical agrocin S1005, effective against *A. tumefaciens* B6-S3, but had become oncogenic as shown by tests on the pea seedlings[7]. By ultracentrifugation and subsequent electron microscopy this oncogenic *A. radiobacter* S1005 (TI Kerr 14) was shown to harbour a large plasmid. The strain had also become sensitive to agrocins 84 and 396, showed an efficient exclusion of phage AP1 and could degrade nopaline. On control pea seedlings inoculated with the recipient strain S1005 *rif str* no tumours were found. From uninoculated pea seedlings no bacteria could be isolated after 6 weeks. From tumours induced by *A. tumefaciens* Kerr 14 but not contaminated with S1005 *rif str* no bacteria could be isolated that were resistant to rifampicin and streptomycin.

These results show that the transfer of virulence as observed by Kerr, rests on a transfer of a large plasmid from the oncogenic strain to the avirulent recipient. By the acquisition of such a plasmid from a taxonomically distant strain, a strain that had been made non-oncogenic by curing of its plasmid as well as a naturally occurring non-oncogenic *A. radiobacter* strain could be rendered oncogenic. Crown gall tumour-inducing ability, ability to degrade nopaline, the exclusion of virulent phage AP1 and the sensitivity to agrocins 84 and 396 are all phenotypic properties coded for by one large plasmid. As tumour-inducing

Table 1 Properties of *Agrobacterium* strains used

Strain	Resistance to 100 μg ml^{-1} rifampicin	Resistance to 300 μg ml^{-1} streptomycin	Ability to produce 3-ketolactose[12]	Ability to grow anaerobically on nitrate-containing medium[5]	Sensitivity to virulent phages of typing set GS1	GS2	GS3	GS5	GS6	GS18	AP1
223	—	—	—	—	—	—	—	—	—	—	—
C58-C1	+	+	+	+	—	+	—	+	+	—	+
C58-C1 (TI 223)	+	+	+	+	—	+	—	+	+	—	+
Kerr 14	—	—	+	+	+	—	+	—	+	+	+
S1005 *rif str*	+	+	+	+	+	+	—	—	+	—	+
S1005 *rif str* (TI Kerr 14)	+	+	+	+	+	+	—	—	+	—	+

Strain	Ability to degrade nopaline	Exclusion of phage AP1	Sensitivity to agrocins 84 and 396	Oncogenicity	Presence of a large plasmid
233	+		+	+	+
C58-C1	—	—	—	—	—
C58-C1 (TI 223)	+	+	+	+	+
Kerr 14	+	+	+	+	+
S1005 *rif str*	—	—	—	—	—
S1005 *rif str* (TI Kerr 14)	+	+	+	+	+

plasmids from two distantly related oncogenic agrobacteria conferred these same properties to their new host, some of these properties may be related to the process of tumour induction. It has been suggested[8] that the receptor sites for agrocin 84 could be part of a specific molecular configuration on the bacterial cell surface necessary for interaction with a proposed specific receptor site on the plant cell wall[9-11]. The correlation between oncogenicity and agrocin 84 sensitivity could however, be simply a result of genetic linkage. The ability to degrade arginine derivatives could be part of a biochemical pathway which, when operating in a plant cell, leads to the production of these amino acids, which in itself could be important in tumour development, or, alternatively or additionally, could sustain further growth of the infecting pathogenic bacteria.

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Received April 2; accepted April 18, 1975.

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Homology of protein structures: proteinase inhibitors

Homology of amino acid sequences is often accompanied by close similarity in the protein tertiary structures. This is illustrated most vividly by the serine proteinases[1] and by the haemoglobin–myoglobin family of haem proteins[2]. The most surprising aspect of the very close similarity in conformations within these protein families is the very large variation in primary structure which is compatible with a given protein conformation; for example, only 3 of the 140 to 150 amino acid residues are invariant in the sequences which give the myoglobin-like conformation.

Examination of this aspect of protein structure in a small, compact protein, with fewer degrees of freedom in its amino acid sequence and its folded structure, such as the bovine pancreatic trypsin inhibitor (BPTI), is useful when considering the relationship between amino acid sequence and protein conformation. The tertiary structure of BPTI has been determined to very high precision by Huber[3] and his colleagues[4]. The amino acid sequences of six other proteins, from sources as diverse as cows, snails, snake toxins and turtle eggs, are homologous with BPTI (Fig. 1). All are proteinase inhibitors. The conformations of none of the homologous proteins have been determined, but consideration of the amino acid sequences in terms of the known tertiary structure of BPTI leaves little doubt that all have very similar conformations.

The numbering of the amino acid residues throughout is that of BPTI, and only residues 1–55 are considered to be of significance. There are no deletions or additions of residues within the primary structure. The BPTI conformation is remarkably compact and rigid, and it is unlikely that any such alterations could be accommodated.

The six cysteine residues, which form three disulphide bonds in BPTI, are invariant. The pairings of cysteine residues in disulphide bonds have been shown to be the same in the bovine colostrum inhibitor[11] and the Russell's viper inhibitor[9], as would be required for the BPTI conformation. Two of the disulphide bonds of BPTI (5–55 and 30–51) are required for maintenance of the native conformation, whereas the third seems to stabilise the protein for its role as a proteinase inhibitor[12].

BPTI has only a small proportion of interior residues, because of its small size, but the aromatic residues 22, 23, 33, 35 and 45, the hydrophobic residues 4 and 21, and Asn-43, the side chain of which participates in three apparently crucial hydrogen bonds in the centre of the molecule, are clearly the most important. All these residues are highly conserved in all the homologous proteins.

Most of the amino acid side chains are on the surface of the molecule and vary widely among the seven proteins. In no case, however, does one of these residues ha

```
                                        1                                   10
Bovine pancreatic inhibitor             Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly Pro Cys Lys Ala Arg
Bovine colostrum inhibitor  Phe Gln Thr Pro Pro Asp Leu Cys Gln Leu Pro Gln Ala Arg Gly Pro Cys Lys Ala Ala
Turtle egg white inhibitor (Lys,Glx,Asx,Gly,Arg)Asp Ile Cys Arg Leu Pro Pro Glu Gln Gly Pro Cys Lys Gly Arg
Snail inhibitor K               Glu Gly Arg Pro Ser Phe Cys Asn Leu Pro Ala Glu Thr Gly Pro Cys Lys Ala Ser
Russell's viper toxin           His Asp Arg Pro Thr Phe Cys Asn Leu Ala Pro Glu Ser Gly Arg Cys Arg Gly His
Black mamba toxin K                     Ala Ala Lys Tyr Cys Lys Leu Pro Leu Arg Ile Gly Pro Cys Lys Arg Lys
Black mamba toxin I            (Gln,Pro)Leu Arg Lys Leu Cys Ile Leu His Arg Asn Pro Gly Arg Cys Tyr Gln Lys


                20                                      30                                      40
        Ile Ile Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Gln Thr Phe Val Tyr Gly Gly Cys Arg Ala Lys Arg
        Leu Leu Arg Tyr Phe Tyr Asx Ser Thr Ser Asn Ala Cys Glu Pro Phe Thr Tyr Gly Gly Cys Gln Gly Asn Asn
        Ile Pro Arg Tyr Phe Tyr Asn Pro Ala Ser Arg Met Cys Glu Ser Phe Ile Tyr Gly Gly Cys Lys Gly Asn Lys
        Phe Arg Gln Tyr Tyr Tyr Asn Ser Lys Ser Gly Gly Cys Gln Gln Phe Ile Tyr Gly Gly Cys Arg Gly Asn Gln
        Leu Arg Arg Ile Tyr Tyr Asn Leu Glu Ser Asn Lys Cys Lys Val Phe Phe Tyr Gly Gly Cys Gly Gly Asn Ala
        Ile Pro Ser Phe Tyr Tyr Lys Trp Lys Ala Lys Gln Cys Leu Pro Phe Asp Tyr Ser Gly Cys Gly Gly Asn Ala
        Ile Pro Ala Phe Tyr Tyr Asn Gln Lys Lys Lys Gln Cys(Glx,Gly)Phe Thr Trp Ser Gly Cys Gly Gly Asn Ser


                                    50
        Asn Asn Phe Lys Ser Ala Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
        Asx Asn Phe Glu Thr Thr Glu Met Cys Leu Arg Ile Cys Glu Pro Pro Gln Gln Thr Asp Lys Ser
        Asn Asn Phe Lys Thr Lys Ala Glu Cys Val Arg Thr Cys Gly Pro Gly Ile Cys Leu.....60 more residues
        Asn Arg Phe Asp Thr Thr Gln Gln Cys Gln Gly Val Cys Val
        Asn Asn Phe Glu Thr Arg Asp Glu Cys Arg Gln Thr Cys Gly Gly Lys
        Asn Arg Phe Lys Thr Ile Glu Glu Cys Arg Arg Thr Cys Val Gly
        Asn Arg Phe Lys Thr Ile Glu Glu Cys Arg Arg Thr Cys Ile Arg Lys
```

Fig. 1 Amino acid sequences of seven homologous proteinase inhibitors: BPTI (ref. 5); bovine colostrum trypsin inhibitor[6]; turtle egg white inhibitor[7]; snail inhibitor K (ref. 8); Russell's viper venom inhibitor[9]; toxins I and K of black mamba venom[10].

large hydrophobic side chain extending into the solvent, which would contradict one of the most obvious rules of protein structure. In certain positions, such as residue 18, non-polar side chains extend to the surface, but they also participate in seemingly important interactions within the structure. Black mamba toxin I has Tyr at residue 15, but this residue is of prime importance for interaction with proteinases.

Glycine residues may attain conformational parameters which are inaccessible for stereochemical reasons to all other amino acid residues; the Gly residues at positions 12, 28, and 37 of BPTI have conformational angles outside the permissible range[4]. In all cases, Gly is invariant at positions 12 and 37, and the model of BPTI further shows that these residues are interior, with no room for a side chain. Residue 28 has been changed, however, to other amino acids in most of the other homologous proteins; these side chains would be on the surface of the molecules, but the conformational angles of this residue in these proteins must be somewhat different from those in BPTI.

Proline residues markedly restrict the conformational angles attainable by the peptide chain (φ must be in the region of $-80°$)[13]. Replacement of Pro by any other amino acid is not affected by such restrictions, but to substitute Pro for any other amino acid without altering the backbone conformation would only be possible if the conformation were compatible with a Pro residue. Such substitutions have occurred in the homologous proteins at positions 11, 19, 25 and 32. The values of φ for these four non-Pro residues in BPTI (refs 3 and 4) range from $-64°$ to $-86°$, which are within the range observed with the four Pro residues of BPTI, $-59°$ to $-89°$. Therefore, the additional Pro residues of the other proteins could be incorporated readily with the BPTI structure.

The amino acid sequences of the six homologous proteins shown in Fig. 1 each seem to be compatible in virtually all respects with the three-dimensional folded conformation of BPTI. The consistency is so striking as to leave little doubt that the native proteins have the BPTI conformation.

The diverse phylogeny of the species in which the seven proteins of Fig. 1 are found, with the usual assumption of evolutionary divergence of structural genes from an ancestor common to all the species, makes it unlikely that the divergence between the primary structures was limited by the rate of mutation. Rather, the residues common to all must have been conserved by natural selection to maintain the native structure, and possibly the function, of the proteins. The physiological functions of the proteins are not known, but, as all are proteinase inhibitors, it is likely that all have functions related to this property. Detailed studies of the proteinase inhibitor properties of BPTI (ref. 14) have indicated that only a few residues in the vicinity of residue 15 participate directly in binding to proteinases. Numerous alterations of the molecule away from this region do not affect the inhibitor activity, as long as the conformation of the molecule is not affected. Therefore, the striking conservation of certain residues in the sequences of Fig. 1 pinpoints those residues crucial for maintaining the folded conformation.

Assumption of any other scheme for the evolutionary origin of the structural genes of the seven proteins (including convergent evolution), using known mechanisms of gene transfer in such organisms, would also require that the striking similarities of certain residues result from requirements of protein conformation.

The six proteins homologous to BPTI presumably are also able to fold into the native conformation on a normal time scale, as does BPTI (ref. 15). These homologous proteins should then be of use in elucidating, both theoretically[16] and experimentally[15], the basis of this remarkable feat.

The apparent concomitance of homology at the levels of both amino acid sequence and three-dimensional conformation requires that a possible primary structure homology also be tested in terms of tertiary structure when possible. The primary structure of an inhibitor of bromelain has recently been suggested[17] to be homologous to the seven proteins of Fig. 1. Consideration of the primary structure of the bromelain inhibitor in terms of the BPTI conformation, however, makes it seem very unlikely that the bromelain inhibitor could have a similar conformation.

The Cys residues of the bromelain inhibitor do not align exactly with those of BPTI, implying insertion and deletion of residues. The bromelain inhibitor has five disulphide bonds, but the four additional Cys residues would not be in relative positions in the BPTI conformation such that disulphide bonds could be formed. The retention of aromatic and hydrophobic residues at positions 4, 22, 23 and 35 in the homologous proteins is not present in the bromelain inhibitor. Large hydrophobic side chains at positions 17, 25, and 28 of the bromelain inhibitor would probably extend into the solvent. The presence of residues other than Gly at positions 12 and 37, and of Pro at position 15, of the bromelain inhibitor would not be permitted with the BPTI conformation. It thus seems very unlikely that the bromelain inhibitor is structurally homologous with BPTI and the six other proteins of Fig. 1.

I thank M. Levitt for discussions and suggestions.

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Received April 2; accepted May 5, 1975.

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Immune exclusion is a function of IgA

PREVENTION of entry of antigen into the systemic circulation through mucous membranes depends partly on the physical integrity of the mucous membranes and the mucous layer and, no doubt, on other nonspecific mechanisms. These systems are, however, not completely effective and there is also a specific acquired capacity for immune exclusion of antigen. For example, everted gut sacs from rats previously fed with an antigen show specific reduced transmission of the antigen, as compared with sacs prepared from animals not fed in this way[1]. Passive transfer *in vivo*, using cell free homogenates of mucosae from antigen-fed animals has also been reported but the mechanism was not identified[2]. One possibility is IgA antibody, the principal immunoglobulin of mucous secretions[3], and this is supported by the demonstration of circulating antibody to food proteins in IgA deficient subjects[4], and the association of allergic disease with IgA deficiency, either permanent[5] or transient[6].

The necessary passive transfer experiments, using pure IgA from the serum or secretions of an immunised animal are difficult. The problems can be avoided by using an IgA myeloma protein from an unimmunised animal. The mouse tumour MOPC-315 secretes an IgA myeloma protein which binds to dinitrophenol (DNP) with affinity well into the antibody range[7] at a concentration in serum of 15-20 mg ml^{-1} (ref. 8). We have demonstrated that serum from unimmunised mice, containing this IgA protein, prevents the absorption through the rat respiratory tract of ^{125}I-labelled human serum albumin to which DNP is conjugated (DNP-^{125}I-HSA) but not ^{125}I-HSA.

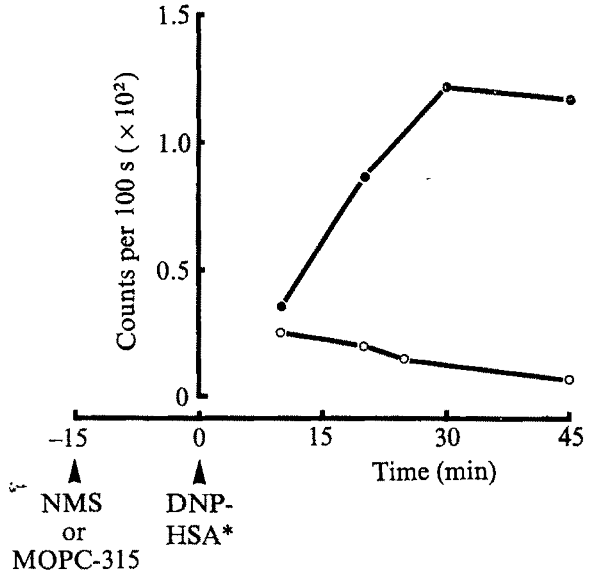

Fig. 1 Radioactivity in the serum of two rats injected into the trachea with ^{125}I-labelled DNP-HSA (DNP-HSA*) at time 0. Fifteen minutes previously the rats had received (also by intratracheal injection) either mouse serum containing MOPC-315 IgA myeloma protein (○) or normal mouse serum (●).

Approximately 8 mm^3 of MOPC-315 tumour were injected into each of 24 BALB/c mice, which were exsanguinated 21 d later. Their sera were pooled, and the IgA concentration found to be approximately 10 times that of normal mouse serum IgA (single radial diffusion using a rabbit anti mouse IgA). HSA (Kabi) was conjugated with DNP by a modification of Porter's method[9], to achieve an average of 4 DNP groups per HSA molecule. DNP-HSA and HSA were labelled with ^{125}I by a chloramine T method[10].

A range of concentrations of MOPC 315 serum (Neat—1:32,000 dilution) was reacted with DNP-^{125}I-HSA (0.25 μg) at 37 °C for 4 h and at 4 °C for 16 h. The mixtures were centrifuged, the supernatants discarded and residual radioactivity counted. Maximum 'counts bound' were obtained with a 1:4,000 dilution of the pooled serum and this was taken as equivalence for this system.

Wistar rats (200–400 g) were anaesthetised with avertin, the trachea exposed and 100 μl of either normal mouse serum or MOPC-315 serum injected into it. After 15 min 6.25 μg DNP-^{125}I-HSA (that is 250-fold less antigen than equivalent for the antigen binding properties of the myeloma serum) were injected into the trachea. The rats were then bled by cardiac puncture at timed intervals and the radioactivity in the serum counted in a Wallac counter.

The time sequence of radioactivity in the serum of a rat which received MOPC 315 serum and another which received normal mouse serum (NMS) are shown in Fig. 1. The rat receiving NMS showed increasing radioactivity, reaching a peak at 30 min, whereas the rat receiving MOPC 315 serum showed a low falling activity only. Similar sequences were found in 6 other rats, three in each group. A clear distinction was shown by 20 min, and anaesthesia for longer than this presented problems, so further work was done at this time. The distribution of radioactivity in the rats was observed by scanning with a Manual Gamma probe, connected to a ratemeter (Nuclear Enterprises Ltd). In both experimental and control groups most of the radioactivity remained in the thorax.

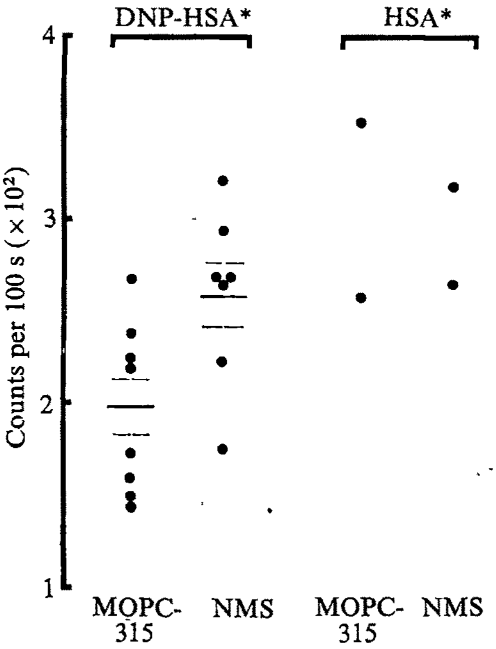

Fig. 2 Serum radioactivity in rats 20 min after intratracheal injections of either ^{125}I-labelled DNP-HSA (DNP–HSA*) or ^{125}I-labelled HSA (HSA*). They had received either mouse serum containing MOPC-315 IgA myeloma protein or normal mouse serum (NMS) into the trachea 15 min before. Mean ± s.e.m. are shown.

In a second experiment, rats were bled only at 20 min. The radioactivity in the serum was rather higher in both groups than in the initial experiments but it was significantly less ($t = 2.54$; $P < 0.025$) in the group receiving MOPC 315 serum than in that receiving NMS (Fig. 2). There was no such obvious difference in the serum radioactivity in four rats receiving ^{125}I-HSA instead of DNP-^{125}I-HSA, after either MOPC-315 or normal mouse serum.

These experiments show that an IgA myeloma protein, injected into the rat respiratory tract, can specifically prevent entry into the blood of antigen for which it has binding affinity. This strengthens the view that IgA may be responsible for this process in both the respiratory tract, in which this is the first evidence of specific immune exclusion, as well, presumably, as in the gut where such a phenomenon has already been demonstrated[2] without the mechanism being established. The evidence that this mechanism is defective in IgA deficient individuals, namely their antibodies to food proteins[4] and allergy[5,6,11], supports the view that IgA is important for this function, but immunodeficiency is often complex and other mechanisms might have been defective in such patients. Limited experiments with IgG rabbit anti-HSA antibody suggest that IgG may also be effective in our system. Walker and his colleagues[12] detected IgG1 anti-BSA antibody in extracts of intestinal mucosa and in intestinal secretions of rats immunised with BSA, so the IgG class may also be important, but the predominance of IgA in the secretions suggests that this is the main mechanism.

The use of the IgA myeloma protein avoids all the usual problems of establishing immunoglobulin class specificity of the observed biological effect, since the animals were not immunised. The negative controls using the DNP-^{125}I-HSA with normal mouse serum and using ^{125}I-HSA with both MOPC 315 serum and NMS establish that it was an effect of IgA and exclude the possibility of nonspecific binding to HSA, a property of many IgA myeloma proteins[13].

The observed antigen exclusion in our experiments was an effect of IgA which probably lacked secretory component. It is likely that normal secretory IgA antibody, with its reported resistance to proteolysis[14], would be much more efficient. It is also possible, however, that the effect was due to polymerised IgA associated with secretory component derived from secretions of the recipient rats. In either case we have shown that IgA can produce this effect, even though the artificial system used may be suboptimal.

It is attractive to suggest that in the gut the complexes of IgA antibody with antigen are retained in the lumen till the antigen is digested. In the respiratory tract they may be swept up by cilliary action, swallowed and similarly digested, although most of the antigen remained in the lungs in our acute experiments. If the adherence of IgA to mucous membranes[15] is important in this system, it is possible that macrophages would be attracted by the chemotactic effect of complement, activated through the alternative pathway[16].

If the function of immune exclusion were defective in an individual capable of eliciting IgE, IgG or cell mediated responses to the antigen, local allergic lung or gut damage or generalised soluble complex disease could occur. Even a transient defect such as in presymptomatic atopic infants[6] could lead to persistent disease, since, once a population of sensitised cells is established, very little subsequent antigen entry would be required to maintain the process. Defects of the system we have described may, therefore, be the basis of many common diseases.

We thank Mr B. Gregory for help with the animals, Dr R. M. E. Parkhouse for a tumour bearing mouse, and the Chest and Heart Association for financial support.

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Received March 7; accepted May 12, 1975.

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Erratum

In the article "Role of macrophages in *in vitro* induction of T-helper cells" by P. Erb and M. Feldmann (*Nature*, **254**, 352; 1975), page 353, column 2, line 3, for presence read absence.

In the article "Centrometric asymmetry and induction of translocations and sister chromatid exchanges in mouse chromosomes" by M S. Lin and R. L. Davidson (*Nature*, **254**, 354; 1975) Figs 1 and 3 were transposed. The captions are correct as they stand.

In the article "Synthesis of ribosomal RNA during sporulation in *Bacillus subtilis*" by D. Testa and R. Rudner (*Nature*, **254**, 632; 1975) Fig. 1 was rotated through 90°. The time scale should have been on the abscissa.

In the article "Crystallisation of troponin-C" by D. Mercola, B Bullard and J. Priest (*Nature*, **254**, 634; 1975) the results in the first sentence of paragraph 4 are not attributable to ref. 12.

reviews

Playing with ideas

Eric Ashby

THE distinguished biochemist Parnas once invited me to look at his collection of offprints. At first there seemed to be nothing unusual about them; all I saw were the familiar rows of labelled boxes. Then Parnas explained what was unusual: the offprints, from hundreds of authors, were catalogued under the names of only five or six biochemists. "When I read a paper", Parnas said, "I recognise in it the ideas of one or other of no more than six creative men —Hopkins, Englehardt . . .—and I classify the paper accordingly".

*The Creative Process in Science and Medicine** quotes a remark by Lewis and Randall (from a treatise on thermodynamics) which is similarly perceptive. The edifice of science is like that of a cathedral built by a few architects and numerous workmen. A scientist does not have to be creative in order to be a valuable member of the scientific community. But the grand design of the edifice of science depends on the creative activities of a mere handful of workers.

What is the secret of this rare creative faculty? In May 1974 C. H. Boehringer Sohn organised a symposium to ask this question. Twenty four people assembled at Kronberg for a couple of days to converse about the creative process in science and medicine. This book records their conversations.

Most unscripted discussions on radio and television are vapid and frothy: they rarely rise to a level which makes it worth while to set them in print. This little book demonstrates that unscripted discussions can be illuminating as well as lively. For two reasons, these conversations are well worth printing: first, some of the contributors are in the front rank of creative scientists (they include four Nobel prizewinners); second, the conversations have been edited with great skill, so that they cleverly combine informality with clarity.

Monod opened the discussions by giving his views about what he called "ingredients" of the creative process, illustrated by the steps of reasoning which went into some of his own classical work on enzyme action. The theme was taken up by Krebs, Popper, Eisenberg, Eigen, Eccles, Tinbergen, and others. Occasionally the distinguished party strayed from the theme or followed some wayward idea like kittens playing with a ball of wool; but in the end a remarkable consensus emerged about the conditions under which the creative process operates.

There are certain ground rules. One should not, for example, entertain any hypothesis unless it is one which "can be falsified even by an imaginary experiment". One needs to have "technical courage"—never be frightened of breaking a rule or switching to a new technique. Orthodoxy, says one speaker, is the enemy of creativity. One has to have the "capacity to pay attention to oddities"; a flair for elegance of thought, a sense of symmetry; something the symposium summed up as "good taste".

There are, too, certain environmental prerequisites. "You cannot be creative", says Eigen, "until you have new experiences". Eccles is even more specific. The experiences have got to be challenging: "Problems with ideas that are in conflict are an important challenge. Conflict is one of the most essential conditions". There is agreement about the necessity for an incubation period—familiar to those engaged on even the most modest kinds of creative work—when the ideas are put out of mind for a time and (sometimes) become illuminated in a fresh and exciting way when they are re-examined. 'Playing with ideas' is an important element in the creative process, and there is some interesting talk as to whether the scientist has something to learn from the way children and animals engage in play.

What is the neurological and anatomical basis for creative thought? Eccles gives a lucid summary of the intricate way in which intentions in the brain are translated into action by muscles. But since uncreative people and even higher animals have similarly intricate neurological 'outfits', this gives no clue to the physical basis of the creative process. Indeed, one of the refreshing impressions one gets from reading this record of the conversations is of the readiness of creative scientists to admit that in the study of human behaviour there is what Popper calls *"Erdenrest"*, "a remainder or residue which is not rational". This is not to say that rational thought must not be pushed to the very limits of human capacity; but it does mean that one must not exclude the possibility that some phenomena lie beyond rational thought, and will remain out of reach whatever advances science makes. Perhaps a better way to put this would be to say that if some human achievements are explained rationally, the achievements themselves vanish. Einstein is reported to have made a remark which illustrates this. Asked whether everything could ultimately be expressed in scientific terms he replied: "Yes, that is conceivable, but it would make no sense. It would be as if one were to reproduce Beethoven's Ninth Symphony in the form of an air pressure curve".

These conversations will not serve as much of a guide to aspirants hoping to acquire creativity. But they will help the ordinary mortal to appreciate more fully how the creative process works in those rare persons lucky enough to have the capacity for it. □

**The Creative Process in Science and Medicine.* (Proceedings of the C. H. Boehringer Sohn Symposium, May 1974.) Edited by Hans A. Krebs and Julian H. Shelley. Pp. xii+138. (Excerpta Medica: Amsterdam; American Elsevier: New York, 1974.) Dfl.52; $21.75.

Solid state and orbital theories

Solid State and Molecular Theory: A Scientific Biography. By John C. Slater. Pp. 357. (Wiley-Interscience: New York and London, March 1975.) £10.25.

JOHN SLATER started research in 1923 and was from the first concerned with atomic, molecular and solid state theory. Nobody could be better qualified to tell the story of the development of these subjects, and this Slater has done in a very personal way.

Of the days before quantum mechanics there are some curious sidelights. On a visit to the Cavendish and Copenhagen he became convinced of the existence of photons, but found that Bohr refused to consider the idea and dissuaded him from publishing—and that still rankles. Then came 1925 and 1926 and the startlingly sudden formulation of quantum and wave mechanics, which Slater describes. Of this period Slater says there seemed to be among theoretical physicists two types: one prosaic, matter of fact and willing to indicate the argument behind what he did; the other like a magician, waving his hands as if he were drawing a rabbit out of a hat. Though admiring—who could not?—the achievements of some of the latter, Slater puts himself in the former category. I would agree, and it is in thorough and detailed work on solids and molecules, a description of which forms the main part of the book, that he has made his greatest contributions. Particularly charming is his account of the early days of many-electron wave functions, when Wigner, Hund and others entered the field with their *"Gruppenpest"*, the pest of group theory, as disgruntled people who had never studied the subject in school described it. By his introduction of the "Slater determinant" he provoked the remark that "Slater has slain the *Gruppenpest*"—and, as he says, his paper was universally popular.

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The account of the application of advanced computers to problems of molecular wave functions occupies the last part of the book. Slater has been involved with this for the last 10 years since he retired from MIT. As he points out, this kind of work had to await the development of the computer, and could not have been done earlier. In this Slater is clearly in his element. His likes and dislikes come out strongly, both as regards persons and methods. He likes a clearly defined problem, complicated enough to be difficult but simple enough for the computer of the day. Approximate methods, valid for even more complicated problems, are less to his taste. Thus the pseudopotential is not mentioned and the work of the English school using such methods is rather summarily dismissed Curiously, for a physicist who has done so much for metal theory, there is no mention of Landau's work on the nature of the Fermi surface. Slater, one feels, draws a line round himself and the computer, and those outside it are the handwaving magicians. But within this circle Slater's achievements are second to none and all his friends and colleagues will hope that his present very successful work will continue for many years. —**Nevill Mott**

Orbital Theories of Molecules and Solids. Edited by N H. March. Pp. xiv+385. (Clarendon: Oxford; Oxford University Press: London; November 1974.) £10.75.

IN 1973 Charles Coulson celebrated his 60th birthday and part of the tribute paid to him by his students and close collaborators is this book. It is essentially a review of the current state of those research fields to which the greater part of his scientific life was dedicated.

The book divides into two distinct areas in spite of all the efforts of the authors to maintain continuity. The solid state part begins with a general survey by Pincherle of the present state of band theory calculations. Then Altmann presents a detailed review of the often misunderstood cellular methods and shows in a convincing way that their neglect is not justified. If those papers stress the orbital concept in its delocalised form the next two redress the balance by considering the atoms. The editor, March, contributes a masterly review of the theory of the potential around atoms and around defects in crystals and shows how many-body effects can be assimilated into what may be considered a typical orbital concept. The different kinds of defect that can occur in crystals of different types is the subject of Lidiard's chapter. He demonstrates, more convincingly than in any other chapter, the fruitful interplay between molecular and solid state ideas. The extent to which this field has been organised and analysed theoretically is impressive.

The molecular half of the book begins with a most useful account by McWeeny of the electronic properties of molecules. He stresses the role of density matrices in unifying and simplifying these properties and although he refers to a large number of spin and magnetic properties the reader is given simple interpretations of their origin. Although Coulson is best known for his molecular orbital work he was prepared to use other theories when they suited his purpose. It is fitting, therefore, that the next chapter, by Balint-Kurti and Karplus on atoms in molecules, should use valence bond ideas to obtain calculated molecular energies in better accord with experiments than simple molecular orbital results could ever give.

The book concludes with two articles which carry the subject into different regions. Murrell presents a careful look at the theory of intermolecular forces and shows that, though caution is needed in some instances, the traditional division into additive components can still be justified despite all the complications of a more sophisticated theory. Craig brings the book back to the theme of molecules and solids with an up-to-the-moment account of the theory of excitions in molecular solids Here, the nature of the free molecule and of its association in the solid, a major interest of Coulson's, are seen to contribute equally to an understanding of the complicated structure of the excited states of molecules in crystals.

The death of Charles Coulson came as a bitter blow to those who looked to him for new research ideas and new insights into old truths. This book which demonstrates both the enduring quality of his own basic ideas and the sound training he gave his students will be a worthy memorial to him. **G. G. Hall**

Cells in animals . . .

Differentiation and Growth of Cells in Vertebrate Tissues. Edited by G. Goldspink. xi+323 (Chapman and Hall: London; distributed in the USA by Halsted Press; December 1974.) £10.30.

As with most multi-author works, this book is largely a heterogeneous collection of the interests of the contributors. The dustcover states that the aim of the book is to "bring to the fore a relatively neglected part of developmental biology . . . namely the development of the cells in different tissues of the body". This the book achieves for seven selected tissues, but only at the expense of continuity and by becoming repetitious. The first and last articles are, however, exceptions in that they are not restricted to any type of cell.

The book is divided into nine chapters, seven of which deal separately with the growth and development of nerve cells, muscle, bone and connective tissue, skin, gonads, blood cells and lymphoid tissue, in that order. It opens with an excellent account by Jane E. Brown and K. W. Jones of the current concepts of differentiation, which effectively explains well established phenomena of differentiative processes in terms of molecular biology. There is an up-to-date discussion of topics like gene activation, transcriptional control and biogenesis of messenger RNA, but one cannot help wondering how long it will be before some of the ideas become outdated, considering the rapid changes taking place in this field.

The second chapter is a very brief account by M. Jacobson of how nerve cells grow and differentiate, much of it dealing with positional specificity of connections in the visual system. The editor's contribution is a well balanced blend on the morphology of developing muscle cells and the control of biosynthesis of muscle proteins. J. J. Pritchard's description of bone and connective tissue is largely at the histological level and so is that of skin by F. J. Ebling, except that the latter takes an evolutionary or comparative approach to development which makes his chapter particularly interesting. Descriptive morphology (at the ultrastructural level) is also the theme of B. Gondos' article on gonads. P. F. Harris gives a comprehensive survey of the origin and maturation of all the major classes of red and white blood cells, with a good selection of electron micrographs. Margaret Manning and J. D. Horton lay more emphasis on function than on morphogenesis in discussing the role of lymphoid cells in immunological defence.

The book ends rather abruptly with D. Bellamy's cursory account of the different theories on ageing and cell death. A discussion of senescence and death in a book on developmental biology is undoubtedly most desirable and it is indeed a pity that in this case it is so superficial. Some of the theories of ageing are complicated or outdated and the brevity makes it especially difficult to sort out firm experimental evidence from vague hypotheses.

This book will be particularly valuable for the postgraduate or postdoctoral research worker who is not yet a specialist in any of the fields covered in the book. It is well presented with a summary and useful bibliography at the end of each chapter. The pleasant print and good photographic reproduction are marred by the fact that about 30 pages of one chapter are upside down—perhaps my copy was an odd one.

J. R. Tata

. . . and plants

The Physical Biology of Plant Cell Walls. By R D. Preston. Pp. xiv+491. (Chapman and Hall: London; distributed in the USA by Halsted Press; September 1974.) £12.00.

THE plant cell wall has been examined using numerous physical, chemical and biochemical techniques. This book concentrates on the results produced by physicochemical analyses. These methods, such as X-ray diffraction, electron microscopy, and the measurement of mechanical properties, show clearly the properties and structure of the microfibrils in the wall but do not indicate much about the matrix materials. Thus, this text is mainly about cellulose and represents a comprehensive and authoritative account of the deposition, organisation, structure and possible mechanisms of the biosynthesis of that important structural polysacharide.

As some of the physical methods used may not be familiar to botanists the author develops very clearly the elementary theory of the techniques and the practical difficulties of their application Each method is discussed, so that the limitations and the pitfalls in the interpretations of the data obtained from the technique can be clearly appreciated. The results are presented as a logical sequence and are critically appraised to show how the ideas and concepts of microfibrillar structure and organisation have developed.

Although the author discusses various conflicting viewpoints and describes the importance and the significance of the different theories, it is usually very clear to which theory the author himself subscribes. But he takes no stand without giving a thorough and detailed argument in its favour.

The book is well illustrated with clear diagrams and numerous electron micrographs. It is well indexed and easy to read. Altogether it represents a very useful and even essential addition to any library connected with a laboratory dealing with plant material and tissues.

D. H. Northcote

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Science Paperback: 412 12640 0 £2.50
Written in a clear straightforward style, with many illustrations, this book is a concise but comprehensive introduction to bacteriophage biology. It covers structure and function, 'growth', survival and death The contribution of bacteriophages to fundamental biological research and their role in medicine and industry are examined. The wide range of microbiological techniques which have led to the discovery of many of the precepts of both bacteriophage and general biology are also discussed.

Chapman & Hall,
11 New Fetter Lane, London EC4P 4EE

nnouncements

wards

he Institution of Mining and Metal-urgy has awarded its **Gold Medal** to **J. Lumsden** for contributions to physical chemistry of extractive metallurgy, particularly the thermodynamics of smelting operations.

The Linnean Society has awarded **Gold Medals** to **P. M. Sheppard, FRS**, and **A. S. Watt, FRS.** The **H. H. Bloomer Award and Medal** (for a distinguished amateur naturalist) goes to **E. S. Edees,** author of *Flora of Staffordshire.*

The National Research Council (Washington, DC) has presented **H. Isbell** with the **Nathan B. Eddy Memorial Award** for research into the problems of drug dependence.

The trustees of the Lady Tata Memorial Trust have made awards to the following: **C. Guibout, J. Phillips, L. Vardimon** and **M-C. Groessens Van-Dyck.**

Appointment

The University of Newcastle upon Tyne has appointed **E. S. Page** and **J. R. O'Callaghan** as pro-vice-chancellors.

Miscellaneous

Chemistry award. The Meldola Medal and Prize (of £100) will be made in 1976 to a British chemist (under age 30) who shows the most promise as indicated by his or her published work brought to the notice of the Royal Institute of Chemistry before December 31, 1975. Further information from: The President, Royal Institute of Chemistry, 30 Russell Square, London WC1B 5DT, UK.

Beilby medal and prize. This award will be made during 1976 to a British scientist (under age 40) in recognition of independent original work of exceptional merit, carried out over a period of years and involving the development and application of scientific principles in chemical engineering, fuel technology or metallurgy, in their modern interpretations. Closing date: December 31, 1975. Further details from: The Convener of the Administrators, Sir George Beilby Memorial Fund, Royal Institute of Chemistry, 30 Russell Square, London WC1B 5DT, UK.

Person to Person

Barnacle research. Graduate student compiling a bibliography of the genus *Balanus* would like to include thesis and dissertation contributions; if any such work known concerning *Balanus* in any way, please send postcard including author, degree, title, year, institution and short summary if possible (M. Landau, New York Ocean Science Laboratory, Montauk, New York 11954).

Astronomical catalogues. Bibliography of catalogues for the period 1951–75 almost completed. Any information, especially concerning obscure or unpublicised work, gratefully received (Mike Collins, Senior Information Scientist, INSPEC, Station House, Nightingale Road, Hitchin, Hertfordshire SG5 1RJ, UK).

Tornadoes and vortices. Scientist researching into the mechanism of funnel clouds wishes to see original film sequences showing tornadoes and other vortices. Could arrange to visit owner's premises to make videotape recordings of suitable material (J. M. Heighes, JOMAR, College Road, College Town, Sandhurst, Berkshire, UK).

New York–London. Wanted September 1, 1975–July 15, 1976. Two bedroom flat convenient for King's College (Strand) to rent or exchange for same on East Side of Manhattan, within walking distance of Rockefeller University, Cornell–New York Hospital, Sloan–Kettering; available immediately (Professor A. Haschemeyer, Hunter College Box 741, New York, New York 10021).

London accommodation. Canadian family of four urgently require accommodation in London area from August 1, 1975 for six months (Dr R. J. Riopelle, Department of Zoology, Room B6, University College, London WC1E 6BT, UK).

There will be no charge for this service Send items (not more than 60 words) to Robert Vickers at the London office The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted No commercial transactions.

International meetings

August 14–20, **Low temperatur physics,** Otaniemi, Finland (LT 14, c/ Low Temperature Laboratory, Helsink University of Technology, SF–0215 Otaniemi, Finland).

August 15–29, **Cosmic rays,** Munich (K Pinkau, Max-Plank-Institute for Extraterrestrial Physics, D–8046 Garching Munich, Federal Republic of Germany).

August 16–23, **Plant pathology,** Munich (Congress Plant Pathology, Biologisch Bundesanstalt, Messeweg 11/13, D 3300 Braunschweig, Federal Republ of Germany).

August 17–22, **Biomedical engineerin** Edinburgh (The Secretariat, Biologic Engineering Society Conference, Princess Margaret Rose Orthopaedic Hospital, Edinburgh EH10 7ED, UK).

August 17–23, **Biodegradation,** Kingston, Rhode Island (Dr R. W. Traxler, Chairman, 231 Woodward Hall, University of Rhode Island, Kingston Rhode Island, 2881).

August 17–25, **Long-term climati fluctuations,** Norwich, UK (Conferenc organiser, World Meteorologica Organisation, PO Box 5, 1211 Genev 20, Switzerland).

August 18–20, **Nitrogen as a water pollutant,** Copenhagen (J. Lonhold Department of Sanitary Engineerin Technical University of Denmark Lyngby, Denmark).

August 18–22, **Phenomena in ionise gases,** Eindhoven, The Netherlands (F. J. de Hoog, Eindhoven Universit of Technology, PO Box 513, Eindhoven, The Netherlands).

August 18–30, **Pacific science,** Vancouver (13th Pacific Science Congres 1975, University of British Columbi Vancouver 8, Canada).

August 19–22, **Cyclotrons and thei applications,** Zurich (Mr W. Joho, Swis Institute for Nuclear Research, 523 Villigen, Switzerland).

August 20–22, **High polymers,** Hamilton, Ontario (J. Prud'homme, Secretary Treasurer, 18th Canadian High Polymer Forum, Department of Chemistr University of Montreal, PO Box 621 Montreal, Quebec, Canada).